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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,382,229**, on April 16, 2002, by **BRUCE SCHMIDT, JAMES KUTNEY and LAWRENCE MAYER**, for "Anhydrovinblastine for the Treatment of Cancer".

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April 28, 2003
Date

Canada

(CIPO 68)
04-09-02

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## ABSTRACT

The present invention is particularly directed to the use of a derivative of vinblastine, 3′,4′- dehydrovinblastine (3′,4′- anhydrovinblastine: AHVB), which differs from vinblastine in that it possesses a double bond at the 3′,4′ position of the catharanthine nucleus rather than the hydroxyl group that is present in the parent structure, in the treatment of cancer.

# ANHYDROVINBLASTINE FOR THE TREATMENT OF CANCER

## TECHNICAL FIELD

The present invention is related generally to the use of antineoplastic vinca alkaloids as antitumour agents. More particularly, the present invention is related to providing use for a derivative of vinblastine, anhydrovinblastine (hereinafter AHVB), as an antineoplastic agent with improved therapeutic properties, demonstrating a significantly higher maximum tolerated dose and less toxicity than its parent and related compounds.

## BACKGROUND OF INVENTION

Due to a high degree of unpredictability, classic techniques of drug development are inventive. Mostly through a process of elimination, a large number of natural products and synthetic chemical compounds are screened for desired effects, using a series of increasingly complex systems, beginning with simple *in vitro* cell-level assays, progressing to animals and finally human clinical trials. But, due to essential characteristics such as adsorption, distribution and metabolism, the initial *in vitro* tests that can not take these features into account could eliminate a powerful drug that does not perform well in such simple systems. The drug could be metabolized to different compounds in animal models than in humans, which may also demonstrate different adsorption or distribution patterns. Or finally, compounds can look very promising all the way through clinical trials, but then demonstrate unpleasant side effects or a high degree of tolerance when used by the human population at large. It is never obvious which compound will continue to look promising as each stage of tests and development are initiated.

Control of tumorous growth has been achieved to a certain degree using oncolytic vinca alkaloids as antitumour agents alone or in combination with other antineoplastic drugs in cancer chemotherapy for more than 20 years. Approximately 30 alkaloids with a wide range of pharmacological activities have been extracted from the Vinca rosea (*Catharanthus roseus*), commonly known as the periwinkle plant. Of these, only vinleurosine, vinrosidine, vinblastine and vincristine possess significant anti-tumour

activity. In particular, vinblastine and vincristine have been used widely as single agents and in combination with outer antineoplastic drugs in cancer chemotherapy. In addition to the naturally occurring alkaloids, some vinca alkaloid analogues have been synthesized by functional transformation or by semisynthetic processes (R.J. Cersosimo, *et al.*, Pharmacotherapy 3:359-274, 1983; P. Mangency, *et al.*, Org. Chem. 44:3765-3768, 1979; R. Maral, *et al.*, Cancer Lett. 22:49-54, 1984).

Chemically, these vinca alkaloids have a dimeric asymmetric structure composed of 2 nuclei linked by a carbon-carbon bond; a dihydroindole nucleus (vindoline), which is the major alkaloid contained in the periwinkle, and the indole nucleus catharanthine (Figure 1). The structural difference between vincristine and vinblastine exists at the R1 position while vinblastine and vindesine differ with regard to the R2 and R3 substituents.

The mode of action of the antineoplastic vinca alkaloids has yet to be completely understood. However, it has been established that the antitumour activity is directly related to the high binding affinity of these compounds for tubulin, the basic protein subunit of microtubules (R.A. Bender and B. Chabner, In: Chabner (ed) Pharmacol. Princ. of Cancer Treat., Saunders, Phil, PA, p. 256, 1982; W.A. Creasey, In: Hahn (ed) Antibiotica, Vol. 2, Springer, Berlin, p. 414, 1979). The consensus is that these agents arrest cell mitosis at metaphase by preventing tubulin polymerization to form microtubules and by inducing depolymerization (R.J. Owellen and C.A. Hartke, Cancer Res., 36:1499-1504, 1976; R.H. Himes and R.N. Kersey, Cancer Res., 36:3798-3806, 1976; R.S. Camplejohn, Cell Tissue Kinet. 13:327-332, 1980). As such, the vinca alkaloids are cell cycle-specific anti-mitotic agents, or spindle poisons. The binding affinity of the vinca alkaloids to tubulin correlates poorly with the relative ability of vincristine, vinblastine and vindesine to inhibit cell growth (R.S. Camplejohn, supra; P.J. Ferguson and C.E. Cass, Cancer Res., 45:5480-5488, 1985). The major difference in anti-tumour activity between these drugs appears, therefore, to relate to their retention in tumour tissue (P. Ferguson, supra; J.K. Horton et al., Biochem. Pharmacol. 37:3995-4000, 1988). In a similar vein, the different toxicity profiles of the vinca alkaloids seems related to tissue uptake and retention properties rather than to inherent tubulin binding affinity. For example, studies have

demonstrated that vincristine is more potent than vinblastine or vindesine in blocking fast axoplasmic transport in nerve cells (S. Ochs and R. Worth, Proc. Am. Assoc. Cancer Res., 16:70, 1975; S.Y. Chan et al., J. Neurobiol. 11:251-264, 1980). In addition, it is taken up into nerves 4 times faster than the other drugs (Z. Iqbal and S. Ochs, J. Neurobiol., 11:251-264, 1980) and exhibits an extended terminal elimination phase of plasma clearance, suggesting a more prolonged exposure to vincristine than to the other vinca alkaloids (R.L. Nelson et al., Cancer Treat. Rev., 7:17-24, 1980).

The *in vitro* and *in vivo* differences observed between the vinca alkaloids are striking given the subtle chemical alterations displayed by the various agents relative to their large, complex molecular structure. For example, vincristine is very effective in treating human rhabdosarcomas transplanted in nude mice whereas vinblastine is not active in this system (N. Bruchovsky et al., Cancer Res. 25:1232-1238, 1965). This difference is obtained simply as a result of the substitution of an aldehyde group for a methyl group at the R1 position. Further, this chemical substitution leads to a shift in the toxicology profile such that peripheral neuropathy (in the absence of hematological toxicity) is dose limiting in humans for vincristine whereas anemia and leucopenia are typically dose limiting for vinblastine (W.P. Brads, Proc. Int. Vincaalkaloid Symposium, 95-123, 1980; S.S. Legha, Med. Toxicol., 1:421-427, 1986). A particularly interesting therapeutic profile has been observed for a new semisynthetic vinca alkaloid named Navelbine™ (vinorelbine, 5'-noranhydroblastine). This compound is less potent than vinblastine and vincristine against murine P388 and L1210 leukemia but is active against cells derived from human lung cancer whereas the other vinca alkaloids are inactive (S. Cros, *et al.*, Seminars in Oncology, 16:15-20, 1989). As well, clinical trials on Navelbine™ support its utility in treating non-small cell lung cancer (A. Depierre *et al.*, Am. J. Clin. Oncol., 14:155- 119, 1991; A. Yokoyama *et al.*, Am. Soc. Clin. Oncol., 11:957, 1992). The toxicity profile of this agent appears similar to that of vinblastine, where hematological toxicities and not neurological side effects are dose limiting.

Vincristine has proved particularly useful as an intravenously administered oncolytic agent in combination with other oncolytic agents for the treatment of various cancers including

central- nervous-system leukemia, Hodgkin's disease, lymphosarcoma, reticulum-cell sarcoma, rhabdomyosarcoma, neuroblastoma, and Wilma tumor. It is for intravenous use only and the intratecal administration is uniformly fatal. Following single weekly doses, the most common adverse reaction is hair loss; the most troublesome are neuromuscular in origin. When single weekly doses of the drug are employed, the adverse reactions of leukopenia, neuritic pain, constipation, and difficulty in walking can occur. Other adverse reactions that have been reported are abdominal cramps, ataxia, foot drop, weight loss, optic atrophy with blindness, transient cortical blindness, fever, cranial nerve manifestations, parehesia and numbness of the digits, polyuria, dysuria, oral ulceration, headache, vomiting, diarrhea, and intestinal necrosis and/or perforation.

Navelbine™ (vinorelbine tartrate) is a novel vinca alkaloid in which the catheranthine unit is the site of structural modification. Its anti-tumour activity is also thought to be due primarily to its ability to interfere with microtubule activity thereby inhibiting mitosis at metaphase through its interaction with tubulin. It is indicated in the treatment of advanced non-small cell lung cancer as a single agent or in combination, administered by intravenous route only. Its side effects include phlebitia or extravasion injury as it is a moderate vasicant. Studies on adverse reactions based on use of Navelbine™ as a single agent indicate Granculocytopenia as the major dose-limiting toxicity, although it was generally reversible and not cumulative over time. Mild to moderate peripheral neutopathy manifested by pareathesia and hypesthesia are the most frequently reported neurologic toxicities, occurring in 10% of patients. Mild to moderate nausea occurs in roughly one-third of patients treated with Navelbine™ with a slightly lesser fraction experiencing constipation, vomiting, diarrhea, anorexia, and stomatitis.
Compounds exhibiting lessened toxic effects with equal or greater chemotherapeutic activity remain to be achieved. Thus, a need remains for a drug providing improved anti-tumour efficacy for the treatment of cancer.

It is, therefore, an object of the present invention to provide a method of treating cancer which comprises administering to a human patient suffering from cancer and in need of

treatment, an amount of AHVB, effective to arrest or significantly slow the progress of the disease.

It is another object of the present invention to provide a method of using AHVB as an antitumour agent, comprising therapeutic amount of the chemical substance of the present invention to arrest tumorous growth.

The above and various other objects and advantages of the present invention are achieved by administration of a derivative of vinblastine, AHVB. Other objects and advantages will become evident from the following detailed description of the present invention.

## SUMMARY OF INVENTION

The present invention is particularly directed to the use of a derivative of vinblastine, 3',4'-anhydrovinblastine (AHVB), which differs from vinblastine in that it possesses a double bond at the 3',4' position of the caranthine nucleus rather than the hydroxyl group that is present in the parent structure, as an antineoplastic agent in the therapeutic treatment of cancer.

One embodiment of the present invention involves the use of 3',4'-anhydrovinblastine, or variants thereof, as an antineoplastic agent in the treatment of cancer.

Another embodiment of the present invention involves the use of 3',4'-anhydrovinblastine, or variants thereof, as an antineoplastic agent, in the treatment of a solid tumor.

Another embodiment of the present invention involves the use of 3',4'-anhydrovinblastine, or variants thereof, as an antineoplastic agent in the treatment of a metastatic tumor.

Yet another embodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of cancer, wherein the concentration of 3',4'-anhydrovinblastine is at significantly higher maximum concentration than therapeutically acceptable concentrations for vincristine or Navelbine™ for use in the treatment of cancer.

Yet a further embodiment of the present invention involves the use of 3',4'-anhydrovinblastine at a dosage concentration of 2.5 to 30 mg/m$^2$ to treat a cancer in a mammal,

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of cervical cancer.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of lung cancer.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of colon cancer.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of breast cancer.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of pancreatic cancer.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of a soft tissue sarcoma.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of a neuroendocrine cancer.

Yet a further emobodiment of the present invention involves the use of 3',4'-anhydrovinblastine as an antineoplastic agent in the treatment of prostate cancer.

## TABLES AND FIGURES

Table 1 shows relative cytotxicity of vincristine, AHVB and Navelbine™ on tumor cell lines.

Table 2 depicts estimates of subacutely toxic dosages of vincristine sulfate, Navelbine™, and AHVB when administered to healthy male Nb rats as a single, intraperitoneal injection.

Table 3 depicts C-4 solid tumour delay in growth data.

Table 4 shows the dosage levels of AHVB administered to patients in a Phase I clinical trial study and describes the associated toxicities at each dosage level.

Table 5 shows the pharmacokinetic parameters of AHVB.

Figure 1 depicts the chemical structure of some vinca alkaloids.

Figure 2 depicts comparison of effects of administering a single intraperitoneal injection, at a subacutely toxic dose, of vincristine, Navelbine™ and AHVB to Nb rats bearing single well-developed, subcutaneous Nb2-U17 tumor transplants on average tumor weight and average weight of the rat as a function of time.

Figure 3 depicts comparison of the effects of administering a single intraperitoneal injection, at a half subacutely toxic dose of vincristine, Navelbine™ and AHVB to Nb rats bearing single well-developed, subcutaneous Nb2-U17 tumor transplants on average tumor weight and average weight of the rat as a function of time.

Figure 4 depicts changes in mean animal wight of BDF1 mice bearing intraperitoneal P388 tumours following i.v. administration of saline, vincristine, Navelbine™ and AHVB.

Figure 5 depicts an example cytotoxicity curve used to estimate the $IC_{50}$ of various vica alkaloids.

Figure 6 depicts P388 anti-tumour activity of selected formulations of vinca alkaloids.

Figure 7 depicts a dose response curve obtained for AHVB when used to treat BDF1 mice bearing P388 tumours.

Figure 8 depicts cytotoxicity curves used to estimate the $IC_{50}$ of AHVB on the cell lines SKOV3 and C-4.

Figure 9 depicts mean tumour weight in grams over time (30 days period) following administration at days 1, 5, and 9, of Navelbine™, bisulphate AHVB, ditartrate AHVB, and control.

Figure 10 depicts measured AHVB serum concentration over a 0 to 72 h time frame for individual patients.

Figure 11 depicts the clearance of AHVB for patients at their respective dose level.

Figure 12 AUC vs. DOSE

Figure 13 Half Life vs. Dose

Figure 14 $C_{max}$ vs. DOSE

Figure 15 is a Goodness of Fit Plot demonstrating the ability of a two-compartment pharmokinetic model to predict the pharmokinetic properties of AHVB.

## DETAILED DESCRIPTION OF THE INVENTION

There are many possible derivatives or variations of vinblastine possible. However, there is no certainty, even to those skilled in the area of anti-cancer drug development, that any such derivatives will be as efficacious or even more efficacious than the parent compound. This takes much testing and experimentation.

The term "variants" for purposes of 3',4'-anhydrovinblastine means any chemical structure that is a derivative of 3',4'-anhydrovinblastine achieved through conservative substitution of side groups, yet still exhibits the same or similar antineoplastic properties as 3',4'-anhydrovinblastine.

Cancers which may be treated using the methods of the invention, include, but are not limited to carcinomas, leukemias (e.g. of the central-nervous system and blood), lymphomas (Hodgkins and non-Hodgkins), sarcomas, melanomas, adenomas, neuroblastomas, nephroblastomas (e.g. Wilm's tumor) and retinoblastomas.

The term carcinoma means a malignant tumor that arises in epithelial tissues found in skin and inner membrane surfaces of the body. Examples of carcinomas (i.e. originating in epithelial tissues such as the skin and inner membrane surfaces of the body), include, but are not limited to cancers such as breast cancer, colon cancer, rectal cancer, esophageal cancer, prostate cancer, lung cancer, stomach cancer, bladder cancer, skin cancer, kidney cancer, pancreatic cancer, ovarian cancer, uterine cancer, cervical cancer, cancer of the vulva, liver cancer, thyroid cancer, aveolar cell carcinoma, basal cell carcinoma, bronchogenic carcinoma, chorionic carcinoma, embyronal carcinoma, giant cell carcinoma, glandular carcinoma, medullary carcinoma, melatonic carcinoma, mucinous carcinoma, oat cell carcinoma, scirrhous carcinoma and squamous cell carcinoma.

The term sarcoma means a malignant tumor that originates in soft tissue of messenchymal origin such as the connective and supportive tissues of muscle, bone, cartilage and fat. Examples of sarcomas (i.e. originating in soft tissues of messenchymal origin such as the connective and supportive tissues of muscle, bone, cartilage and fat), include, but are not limited to cancers such as Kaposi's sarcoma, alveolar soft part sarcoma, bone cancer, botryoid sarcoma, endometrial sarcoma, giant cell sarcoma, osteogenic sarcoma, reticulum cell sarcoma and spindle cell sarcoma, rhabdomyosarcoma and lymphosarcoma.

**Characterization of AHVB Anti-tumour Activity *In Vitro***

Cytotoxicity experiments on AHVB were performed as direct comparisons with vincristine and Navelbine™ in order to assess its inherent antineoplastic profile against a variety of tumour cell types relative to other relevant vinca alkaloids. The cytotoxicity of AHVB was investigated *in vitro* against a panel of tumour cell lines of varying lineage in order to determine the specificity of its antitumour activity with respect to cell type. The tumour lines studied were P388 lymphocytic leukemia (a murine lymphocytic leukemia), Noble (Nb) rat U17 lymphoma, MCF7 human breast carcinoma, H460 human non-small cell lung carcinoma, K562 human erythrokeukemia and LS180 human colon carcinoma based on established NCI *in vitro* new anti-cancer drug cytotoxicity screening protocols.

Standard dose response cytotoxicity assays (R. Mosmass, J. Immunol. Meth., 65:55-64, 1983) were utilized to determine the IC50 (drug concentration required to induce 50% inhibition of tumour cell growth) for vincristine, Navelbine™ and AHVB. The results are presented in Table 1. The indicated cell lines were obtained from either the ATCC or NCI tumour repository and were cultured in tissue culture media by standard techniques well known to those skilled in the art, prior to dilution to a defined cell concentration required for the studies in 96 well plates.

A wide range of drug concentrations were exposed to tumour cells growing at log phase in 96- well microtitre plates. Cell concentrations depended on the cell line as well as the

length of time to be cultured. Typically, P388 cells were plated at a concentration of 30,000, 2,000 and 750 cells per well for studies lasting 1, 3 and 7 days, respectively. MCF7 cells were plated at a concentration of 7,000 and 1,500 cells per well for studies lasting 3 and 7 days, respectively. H460 cells were plated at a concentration of 2,500 and 1,000 cells per well for studies lasting 3 and 7 days, respectively. K562 cells were plated at a concentration of 1,500 and 10,000 cells per well for studies lasting 1 and 3 days, respectively. LS180 cells were plated at a concentration of 5,000 and 20,00 cells per well for studies lasting 3 and 7 days, respectively. After plating all cell lines were incubated ($CO_2$ incubator at 37°C, 5% $CO_2$) for 24 hours prior to addition of the cytotoxic agent (See Table 1).

**RELATIVE CYTOTOXICITY OF VINCRISTINE, AHVB AND NAVELBINE™ ON TUMOR CELL LINES**

| CELL LINES | TYPE | EXPOSURE TIME (days) | DRUG $IC_{50}$ (nM) | | |
|---|---|---|---|---|---|
| | | | VINCRISTINE | NAVELBINE | AHVB |
| P388 | murine leukemia | 1 | 11.0 ><3.6 | 20.0 ><0.0 | 140.0>< 3.0 |
| | | 3 | 1.0 ><0.3 | 0.7><0.3 | 15.0 >< 8.7 |
| | | 7 | 2 | 2.5 | 20 |
| MCF 7 | human breast | 1 | N.D. | N.D. | N.D. |
| | | 3 | >2500 | >2500 | >2500 |
| | | 7 | 2.6 ><1.6 | 2.6 ><1.6 | 31.3 >< 12.4 |
| H460 | human lung | 1 | N.D. | N.D. | N.D. |
| | | 3 | 3.5 | 0.3 | 10 |
| | | 7 | 2.5 | >0.5 | 5 |
| K562 | human erythro-leukemia | 1 | >50.0 | >50.0 | >50.0 |
| | | 3 | 1.5 ><0.4 | 2.5 ><2.2 | 18.8 >< 8.8 |

| | | | | | |
|---|---|---|---|---|---|
| | | 7 | N.D. | N.D. | N.D. |
| LS180 | human colon | 1 | N.D. | N.D. | N.D. |
| | | 3 | >50.0 | >50.0 | >50.0 |
| | | 7 | 1.5 | 0.5 | 17.5 |

Table 1

Subsequently the plates were incubated for the indicated time period. At specified times, cells were washed and subsequently exposed to the dye inclusion marker MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium), which accumulated into viable cells. MTT was added to the cells at a final concentration of 50 µg per well. After a 4 hour incubation, the cells were washed free of media and unreacted MTT, prior to addition of DMSO which was required to solubilize the insoluble formazan precipitate that formed in viable cells. After the sample was mixed through repeated pipetting, the coloured product was measured using a plate reader operating at 570 nm. The absorbance values obtained for cells cultured in the absence of drug was assumed to represent 100% viability. Experiments were repeated to substantiate any differences noted between AHVB and other vinca alkaloids.

**Characterization of AHVB Antitumour Activity *In Vivo***

Evaluation of *in vitro* cell cytotoxicity was followed by studies regarding the antineoplastic activities of AHVB in three *in vivo* rodent models. Thus, anti-tumour activity of AHVB was determined using a rat solid tumour model (U17 lymphoma), the murine P388 tumour model (R. Noble, et al., Cancer Res., 37:1455-1460, 1977; P.W. Gout et al., Biochem Cell Biol., 64:659-666, 1986), and a H460 SC Tumour mouse model.

The U17 cell line was originally derived from a transplantable malignant lymphoma that arose spontaneously in male Noble rats (British Columbia Cancer Research Centre Joint Animal Breeding Facility with parents obtained from the National Institutes of Health, Bethesda, MD). The cell line is prolactin dependent and can readily be cultured *in vitro*.

U17 derived solid tumours are generated by subcutaneous injection (via the trocar method) of a small ($2mm^2$) piece of tumour tissue obtained from male Noble rat. Tumour tissue used for the implants arose two weeks after injection of $5 \times 10^6$ U17 cells (from culture) subcutaneously in the nape of the neck. For assessing the anti-tumour activity of AHVB, tumour bearing animals (2-4 gm tumours) were given a single treatment of drug and tumour size was measured as a function of time following treatment. The anti-tumour activity was assessed at a series of different doses in order to determine the maximum therapeutic dose of AHVB. Comparative studies between vincristine, vinblastine and AHVB were performed. For these studies anti-tumour activity was determined at the maximum therapeutic dose of each drug.

Antitumour studies on mice focussed, in one case, on the P388 leukemia model. This is a standard NCI model for evaluation of new anti-cancer agents and it has been demonstrated to be sensitive to treatment with vinca alkaloids. This is an ascitic tumour model that was generated by intraperitoneal inoculation of $1 \times 10^6$ P388 cells (derived from culture, with an original cell line obtained from the NCI tumour repository) in BDF1 mice (Charles Rivers). One day after tumour cell inoculation, mice were treated with a single intravenous injection of drug. Animal weight was monitored daily and tumour progression was measured as an increase in animal weight and through estimation of survival time. Therapy was described by a decrease in tumour progression and an increase in survival time relative to an untreated control group. Initial studies established the maximum therapeutic dose for AHVB. Subsequently comparative studies with vincristine and Navelbine™ were initiated where animals were treated with each drug at the maximum therapeutic dose.

The Canadian Council on Animal Care Guidelines were strictly adhered to and all animal protocols employed were approved by the Animal Care Committees of UBC and the BCCA. Animals were evaluated twice daily for any signs of stress (tumour or drug related) and if an animal appeared to be suffering (excessive weight loss or gain, lethargy, scruffy coat, etc.) than the animal was terminated.

**Identification of Maximum Tolerated Dose of AHVB**

Range-finding acute (14 day), single dose toxicity studies were performed in healthy male Nb rats in order to determine the maximum tolerated dose of vincristine sulfate, Navelbine™ and AHVB when administered as a single, intraperitoneal injection in these rodents (see Table 2).

| Drug | Dose (mg/kg) | Mortality (surviving rats/injected rats) |
|---|---|---|
| 1 ml Saline pH 4.3 | n/a | 35856 |
| Vincristine sulfate | [illegible] | [illegible] |
| | 0.7 | 35856 |
| | [illegible] | [illegible] |
| | 0.5 | 35856 |
| Navelbine™ (Vinorelbine tartrate) | [illegible] | [illegible] |
| | 5 | 0 |
| | [illegible] | [illegible] |
| | 2 | 35856 |
| | [illegible] | [illegible] |
| Anhydrovinblastine | 10 | 0 |
| | [illegible] | [illegible] |
| | 4.4 | 0 |
| | [illegible] | [illegible] |
| | 3 | 35856 |

Table 2: Estimation of subacutely toxic dosages of vincristine sulfate, Navelbine™, and AHVB when administered to healthy male Nb rats as a single, intraperitoneal injection.

To this end, healthy non-tumour bearing male Nb rats (weight range 333-399 grams) were divided in groups of 3 animals. Each group was used to test one drug at one dosage. In a group, each animal received one intraperitoneal injection at a particular dose, as indicated in Table 2. The volumes within which the drugs were administered depended on the concentration of the drug solution (in saline) and the weight of the animals, and ranged from 0.1 - 1.0 ml. Saline was used as a control. The highest dose of each drug which allowed survival of all animals in a group (3 out of 3) was taken as the subacutely toxic dosage for the drug, i.e. 0.7 mg/kg for vincristine, 2.0 mg/kg for Navelbine™ and 3.0 mg/kg for AHVB.

The health of the animals was assessed by daily weight measurements in addition to behavioural indications of stress. Animals continued to be monitored throughout the complete 14 day study period. Animals were euthanized in the event of signs of severe stress or weight loss in excess of 20%. All animals were necropsied at the end of the study period or at the time of premature euthanasia. Once weight loss in excess of 20 % or

premature animal death was noted at a dose level, the dose was decreased until the weight loss nadir was less than 20% and no premature animal deaths were observed.

**Studies in the Rat U17 Lymphoma Model**

Cultures of the non-metastatic, pre-T Nb2 lymphoma line originally developed at The University of British Columbia and designated Nb2-U17 (Anticancer Research 14:2485-2492, 1994), and are available from the British Columbia Cancer Research Centre. Cells from exponentially growing Nb2-U17 suspension cultures were injected subcutaneously into methoxyflurane- anesthetized, mature male Nb rats (5 rats; 310 - 380 grams; 5 x $10^6$ cells/rat in 1 ml of culture medium) at the nape of the neck using a 1.5" 20-gauge needle. At about 3 weeks, when the tumours reached a size of 4 - 7 cm (length + width), the animals were sacrificed and the tumours used for transplantation as described below.

A tumour from a rat was excised, minced and the tumour tissue was put into trocars (2", 13 gauge). The tissue samples were implanted subcutaneously in the nape of the neck of methoxyflurane-anesthetized male Nb rats (248 - 404 grams; 1 trocar per rat). This procedure was repeated 5 times to get a total of 60 tumour-bearing rats to be used for efficacy studies of the 3 drugs.

When the tumours were well established (1.5 - 2 weeks later), three separate groups of 20 rats, as closely matched as possible in terms of both tumour weight and rat weight, were selected for administration of the three test articles (i.e. one group for each test article).

Vincristine was administered to rats weighing 281 - 384 grams, bearing tumours weighing 6.3 - 16.3 grams. Navelbine™ was administered to rats weighing 274 - 389 grams bearing tumours weighing 9.1 - 23.3 grams. AHVB was administered to rats weighing 303 - 400 grams, bearing tumours weighing 7.9 - 25.9 grams. Tumour weights were estimated using the hemi-ellipsoid model (weight in grams = length x depth x $\pi/6$ in cm).

The oncolytic effects of each of the three drugs were assessed at a subacutely toxic dose, determined for each drug in preliminary studies using non-tumour-bearing, mature male Nb rats, i.e. 3.0, 2.0 and 0.7 mg/kg for AHVB, Navelbine™ and vincristine, respectively as illustrated in Figure 2. In addition, each drug was assessed at 50% and 25% of its subacutely toxic dose. Five tumour-bearing rats were used to evaluate the effect at each dose level. The drugs were administered intraperitoneally as a single bolus in a volume of 0.19 - 0.31 ml, as indicated by the weight of the animals. To this end, drug preparations were diluted to appropriate concentrations using sparged saline adjusted with acetic acid to pH 4.2. For each drug, a group of 5 control rats received an intraperitoneal injection of the equivalent amount of saline (pH 4.2). The tumour-bearing rats were organized in the following groups:

| Group | Drug/Saline | Dose(mg/kg) |
|---|---|---|
| 1 | saline | - |
| 2 | AHVB | 3.0 |
| 3 | AHVB | 1.5 |
| 4 | AHVB | 0.75 |
| 5 | saline | - |
| 6 | Navelbine™ | 2.0 |
| 7 | Navelbine™ | 1.0 |
| 8 | Navelbine™ | 0.5 |
| 9 | saline | - |
| 10 | vincristine | 0.7 |
| 11 | vincristine | 0.35 |
| 12 | vincristine | 0.175 |

Following administration of the test articles, the animals; weight and tumour size (using calipers) were determined daily until the tumour reached an estimated weight of 35 grams, or started to ulcerate, at which times the animals were sacrificed (by carbon dioxide inhalation) and subjected to necropsy. Animals were also monitored at least daily for signs

of stress for the full length of the study. Animals manifesting severe symptoms of stress (rapid weight loss, panting, hunched posture, scruffy coat) were also sacrificed and a necropsy performed.

Anhydrovinblastine Sulfate (3',4'-dehydrovinblastine) was obtained from the British Columbia Cancer Agency (BCCA), Investigational Drug Section. Vincristine Sulfate (Sulfate of 22-oxovincaleukoblastine) was obtained from David Bull Laboratories Ltd., Australia. Navelbine™ (vinorelbine tartrate; 3',4'-didehydro-4'-deoxy-C'-norvincaleukoblastine-di-L-tartrate) was purchased from Burroughs Wellcome Inc., Canada; 0.9% Sodium Chloride Injection USP, pH 4.2 was purchased from Baxter.

The methodology involving animals was approved by the BCCA's Institutional Animal Care Committee (IACC) at UBC prior to conducting the studies (Animal Care Certificate No. A94- 1602). During the study the care, housing and use of animals was performed in accordance with the Canadian Council on Animal Care Guidelines.

The results of the efficacy studies are given in Figures 2 - 3. Figures 2 - 3 present averages of data from 5 or fewer animals.

The effect of administering a single intraperitoneal, subacutely toxic dose of AHVB, Navelbine™ and vincristine on the size of single, well-established Nb2-U17 lymphoma transplants (average weight 10 - 13 grams) and the weight of the animals, as a function of time are demonstrated in Figure 2. Whereas the tumours in the control animals continued to increase in size to an average weight of about 40 grams in 6 days, the tumours in the drug-treated animals in each case regressed to essential non-palpability within 5 days of drug administration. After day 10, recurrence of tumours in Navelbine™- and AHVB-treated animals occurred to about the same extent. In contrast, recurrence of tumours was not observed in vincristine-treated animals (not even on day 29). Figure 2 also shows that the animals lost weight following drug administration. However, most of the weight was regained after about 17 days. As controls for each drug, Nb2-U17 tumour transplant-bearing rats injected with saline were used. For each of the six groups five

animals were used. Vincristine sulfate (0.7 mg/kg) was administered in a volume of 0.20 - 0.23 ml to rats weighing 281 - 331 grams bearing tumours weighing 7.6 - 14.2 grams. Navelbine™ (2.0 mg/kg) was administered in a volume of 0.24 - 0.31 ml to rats weighing 297 - 389 grams bearing tumours weighing 11.5 - 13.7 grams. AHVB (3.0 mg/kg) was administered in a volume of 0.20- 0.24 ml to rats weighing 314 - 374 grams bearing tumours weighing 8.2 - 14.2 grams. Vincristine sulfate controls: saline was administered in a volume of 0.21 - 0.26 ml to rats weighing 294 - 370 grams bearing tumours weighing 9.4 - 14.6 grams. Navelbine™ controls: saline was administered in a volume of 0.25 - 0.29 ml to rats weighing 310 - 365 grams bearing tumours weighing 9.5 - 18.2 grams. AHVB controls: saline was administered in a volume of 0.19 - 0.25 ml to rats weighing 303 - 400 grams bearing tumours weighing 7.9 - 16.6 grams. The efficacies of each drug were determined separately at three different dosages versus a control.

Figure 3 shows the anti-tumour effects of the three drugs at 50% of their individual maximum tolerated doses. The data show that Navelbine™ was less potent than AHVB which in turn was less potent than vincristine.

Nb2-U17 tumour transplant-bearing rats injected with saline were used as controls. For each of the six groups five animals were used. Vincristine sulfate (0.35 mg/kg) was administered in a volume of 0.23 - 0.27 ml to rats weighing 327 - 384 grams bearing tumours weighing 6.4 -13.4 grams. Navelbine™ (1.0 mg/kg) was administered in a volume of 0.24 - 0.28 ml to rats weighing 296 - 351 grams bearing tumors weighing 9.1 - 14.1 grams. AHVB (1.5 mg/kg) was administered in a volume of 0.20 - 0.23 ml to rats weighing 308 - 359 grams bearing tumors weighing 9.7 - 19.5 grams. Vincristine sulfate controls: saline was administered in a volume of 0.21 - 0.26 ml to rats weighing 294 - 370 grams bearing tumours weighing 9.4 - 14.6 grams Navelbine™ controls: saline was administered in a volume of 0.25 - 0.29 ml to rats weighing 310 - 365 grams bearing tumours weighing 9.5 - 18.2 grams. AHVB controls: saline was administered in a volume of 0.19 - 0.25 ml to rats weighing 303 - 400 grams bearing tumors weighing 7.9 - 16.6 grams. The efficacies of each drug were determined separately at three different dosages

versus a control. In Figure 3, results of the three drugs at equivalent, i.e. half subacutely toxic, dosages are compared. The controls in Figure 3 are the same as in Figure 2.

**Studies in the Murine P388 Model**

A cytotoxicity curve was generated to estimate the $IC_{50}$ of vincristine, Navelbine™ and AHVB in the murine P388 cell line (see Figure 5). In this study, P388 cells derived from an ascitic tumour grown in BDF1 were first separated from red cells employing Ficoll-Paque. Isolated white cells were washed twice then placed in serum containing tissue culture media ($1 \times 10_5$ cells per ml of RPMI 1640 supplemented with L-glutamine, penicillin, streptomycin and 10% fetal bovine serum) and cultured for 2 hours. All non adherent cells were collected and that cell population was defined as P388 cells and used for cytotoxicity assays 24 hours later. Cytotoxicity assays were performed as described in the the section entitled Characterization of AHVB Anti-tumour Activity *In Vitro*. The drug concentrations used are indicated on the X-axis. Vincristine is represented by the filled circles, Navelbine™ by the filled triangles and AHVB by the filled squares.

The *in vivo* anti-tumour activity of AHVB was compared to that of vincristine, Navelbine™ in the BDF1-murine P388 model in the procedure as follows. P388 cells were derived from the ascities of previously injected female BDF1 mice (19 - 21 grams) P388 cells, from the NCI tumour repository were inoculated directly into mice. The cells arrive from NCI frozen in 1 ml aliquots. These samples were thawed rapidly at 37°C and subsequently injected (within 1 hour) intraperitoneally into two mice, 0.5 ml per mouse. One week (7 days) after inoculation, the tumour cells were harvested by removing peritoneal fluid using a sterile syringe with a 22 gauge needle. The cells, pooled from two animals, were counted using a heamocytometer, diluted (RPMI media) to a concentration of $2 \times 10^6$ cells/ml and 0.5 ml was then re-injected into each of two BDF1 mice. Remaining cells were washed and placed into a DMSO containing media and frozen (in freezer packs that cool at a defined rate). This process was repeated weekly over a 2- week period. Cells used for anti-tumour studies were collected from the third passage to the 20th

passage. After the 20th passage the cells were no longer used for experimental studies. Newly established cells were derived from the frozen cells prepared as described above.

Groups (five mice per group) of female BDF1 mice (Charles Rivers, Canada) were injected (intraperitoneal) with $10^6$ P388 cells (as described above). One day after tumor cell inoculation, the mice were given a bolus intravenous injection of indicated drug via the lateral tail vein. Control groups were injected with saline. Free drug samples were prepared on the day of injection such that the final concentrations were sufficient to deliver the indicated drug dose in a volume of 200 µl. All dilutions were made using 0.9% Sodium Chloride Injection USP. The mice were briefly (less than 30 sec.) restrained during intravenous injections. Dilation of the vein was achieved by holding the animals under a heat lamp for a period of between five and ten minutes. Following administration of the test articles, animals were weighed daily for fourteen days and monitored for signs of stress twice daily for the first 14 days (once daily on weekends) and once daily for the remainder of the study. Severely distressed animals were terminated by $CO_2$ asphyxiation and the time of death was recorded to occur on the following day. Although complete dose titrations were completed for each drug, the data shown in Figure 6 is that obtained after administration of the free drugs at their maximum tolerated dose. This was 3, 40 and 40 mg/kg for vincristine, Navelbine™ and AHVB, respectively.

Figure 4 presents the results of a study demonstrating vinca alkaloid induced weight loss following a single intravenous injection of the indicated drug at the maximum tolerated dose (see Figure 6). These data were obtained as part of the study detailed in Figure 6. After treating mice (bearing the P388 tumour) with a single dose of the indicated drug, animals were examined twice daily for the first 14 days (once daily on weekends). Mean body weight was determined daily over this time period and the results are shown in Figure 4. Weight gain in the control is an indication of tumour progression. Results indicate that AHVB, administered at 40 mg/kg, is the least toxic of the three drugs evaluated.

The dose response curve obtained for AHVB when used to treat BDF1 mice bearing P388 tumours is presented in Figure 7. The studies were conducted as described for Figure 6.

The maximum tolerated dose of AHVB (40 mg/kg) as specified in these studies reflects a very acute (within 1 hour) toxic reaction that limits further dose excalatin for i.v. administration of AHVB. This contrasts the more prolonged toxicity observed for Navelbine™ at its maximum tolerated dose and suggests that an ability to circumvent the acute toxicity of AHVB could lead to significant increases in its maximum tolerated dose.

Based on observation of the *in vitro* drug screen studies, it is surprising that AHVB would perform well as an antineoplastic agent for use in cancer therapy. The *in vitro* tests indicate that AHVB is consistently 10 to 15 fold less active on per molar basis (Table 1 and Figure 5) than vincristine and Navelbine™. These results suggest that AHVB would not perform well as an anti-tumour agent. However, in an efficacy study, also employing the P388 cell line (see Figure 6), the anti-tumour activity of AHVB at the maximum tolerated dose (40 mg/kg, single i.v. injection) is significantly better than that observed for vincristine (administered at the maximum tolerated dose of the free drug of 3 mg/kg). Improved anti-tumour activity, in this case, is measured by the number of long term survivors (>60 days). It is important to stress that, for this example, AHVB is approximately 10 times less toxic (on a weight basis) than vincristine. Therefore, 10 times more drug can be given and it is at this dose that improvements were observed in the long term survival of animals with P388 tumours. When compared to Navelbine™, the *in vivo* results are even more surprising as the maximum tolerated dose of the two drugs in animals bearing P388 tumours are about the same (40 mg/kg).

Figure 8 shows the cytotoxicity of AVHB on SK0V3 cells and C-4 cells with a 3 day incubation. The $IC_{50}$s for the SK0V3 and C-4 cells were 4.0 M and 0.02 M respectively. Both cell lines were obtained from the ATCC and grown using standard growth techniques and medium as described above. The $IC_{50}$s were determined through standard cytoxicity assays described above, with each well containing approximately $10^4$ cells.

**Studies in the H460 SC Tumour Mouse Model**

Cultures of H460 Human Lung cells are available from the British Columbia Cancer Research Center. Cells were injected subcutaneously twice into mature male Rag-2 mice (24 mice, 1 x $10^6$ cells/mouse) using a 26-gauge needle. The H460 cells were suspended in a Hank=s Balanced Salt Solution without calcium. Tumours were allowed to form in the mice for 11 days.

When the tumours were well established, four separate groups of mice, were selected for administration of the three test articles (*i.e.* one group for each test article of AHVB bisulphate, AHVB ditartrate, and Navelbine™) and one control.

AHVB bisulphate and ditartrate, and Navelbine™ were solubilized using 5% dextrose saturated with Argon. Both of these articles were at a concentration of 20 mg/ml. Any dose dilutions were made with 5% dextrose.

The articles were administered intravenously on the days 1, 5 and 9, as were controls of 5% dextrose. Body weights and tumour measurements with calipers were taken every day for the first 10 days and then every other day for the remainder of the study.
Following administration of the test articles, the animals; weight and tumour size (using calipers) were determined daily for the first 10 days and then every other day for the remainder of the study. If the tumour size reached 1 gram in weight or the tumour started to ulcerate, the animals were sacrificed (by carbon dioxide inhalation) and subjected to necropsy. Animals were also monitored at least daily for signs of stress for the full length of the study. Animals manifesting severe symptoms of stress (rapid weight loss, panting, hunched posture, scruffy coat) were also sacrificed and a necropsy performed.

Anhydrovinblastine Sulfate (3',4'-dehydrovinblastine) was obtained from the British Columbia Cancer Agency (BCCA), Investigational Drug Section. Navelbine™ (vinorelbine tartrate; 3',4'-didehydro-4'- deoxy-C'-norvincaleukoblastine-di-L-tartrate) was purchased from Glaxo/Burroughs Wellcome Inc., Canada.

The methodology involving animals was approved by the BCCA's Institutional Animal Care Committee (IACC) at UBC prior to conducting the studies (Animal Care Certificate No. A94- 1602). During the study the care, housing and use of animals was performed in accordance with the Canadian Council on Animal Care Guidelines.

The results of the efficacy studies are given in Figure 9 and present averages of data from 6 or fewer animals. Each mouse in a given article group had two subcutaneous tumours on its back. Each tumour was measured in length and width and the volume of each tumour was calculated by $(L \times W)^2/2$. The two tumour volumes were then averaged. The volume averages of all the mice/group were averaged to yield a mean for the single date point appears on the graph in Figure 9. The calculation was performed each day the tumours were measured. The standard deviation of the mean and the standard error of the mean were calculated with the error bars appearing in the graph in Figure 9.

**Studies in the C-4 (Cervical) Solid Tumour Model**

Cultures of C-4 Human Cervical Carcinoma cells are available from the British Columbia Cancer Research Centre. Cells were injected subcutaneously twice into mature male Rag-2 mice (24 mice, 1 x $10^6$ cells/mouse) using a 26-gauge needle. The C-4 cells were suspended in a Hank=s Balanced Salt Solution without calcium. Tumours were allowed to form in the mice for 31 days.

When the tumours were well established, four separate groups of mice, were selected for administration of the three test articles (*i.e.* one group for each test article of AHVB bisulphate, AHVB ditartrate, and Navelbine™) and one control.

AHVB bisulphate and ditartrate, and Navelbine™ were solubilized using 5% dextrose saturated with Argon. These articles were administered at doses of 20 mg/Kg I.V. Any dose dilutions were made with 5% dextrose.

The articles were administered intravenously on the days 1, 5 and 9, as were controls of 5% dextrose. Body weights and tumour measurements with calipers were taken regularly over the period of the study of 69 days.

Following administration of the test articles, the animals; weight and tumour size (using calipers) were determined regulary over the period of the study. If the tumour size reached 1 gram in weight or the tumour started to ulcerate, the animals were sacrificed (by carbon dioxide inhalation) and subjected to necropsy. Animals were also monitored at least daily for signs of stress for the full length of the study. Animals manifesting severe symptoms of stress (rapid weight loss, panting, hunched posture, scruffy coat) were also sacrificed and a necropsy performed.

Anhydrovinblastine Sulfate (3',4'-dehydrovinblastine) was obtained from the British Columbia Cancer Agency (BCCA), Investigational Drug Section. Navelbine™ (vinorelbine tartrate; 3',4'-didehydro-4'- deoxy-C'-norvincaleukoblastine-di-L-tartrate) was purchased from Glaxo/Burroughs Wellcome Inc., Canada.

The methodology involving animals was approved by the BCCA's Institutional Animal Care Committee (IACC) at UBC prior to conducting the studies (Animal Care Certificate No. A94- 1602). During the study the care, housing and use of animals was performed in accordance with the Canadian Council on Animal Care Guidelines.

The results of the efficacy studies are given in Table 3 and present averages of data from 6 or fewer animals. Each mouse in a given article group had two subcutaneous tumours on its back. Each tumour was measured in length and width and the volume of each tumour was calculated by $(L \times W)^2/2$. The two tumour volumes were then averaged. The volume averages of all the mice/group were averaged to yield a mean for each single date point. The calculation was performed each day the tumours were measured.

Navelbine™ tumours reached their observable >growth threshold=at day 41 and continued to grow steadily whereas the AHVB ditartrate reached the threshold on day 55. The

tumour treated with AHVB bisulphate showed negligible tumour growth through day 69. Navelbine™ had an 84% delay in growth in the tumour, AHVB ditartrate had an extended delay of 106%, and AHVB bisulphate exhibited a marked delay in tumour growth of greater than 209%. Tumour growth did not reach the observable growth threshold over 70 days. This data is found in Table 3 below.

Table 3: Solid Tumour Delay in Growth Data

| EXPERIMENT | DOSE 20 mg/kg IV days 1,5,9 | INITIAL GROWTH (day) | | % DELAY IN GROWTH (DIG) |
|---|---|---|---|---|
| | | TOTAL | OF EXPT. | |
| C-4eff1 | Control (Saline) | 32 | 2 | |
| | Navelbine™ | 59 | 29 | 84 |
| | AHVB Bisulphate | 99 | 69 | 209 |
| | AHVB Ditartrate | 66 | 36 | 106 |

Taken together, the results presented here show that AHVB has significant and unique pharmacological properties *in vivo* that lead to significant improvements in *in vivo* antitumor efficacy relative to other vinca alkaloids such as vincristine and Navelbine™. These results are unique and new in that the *in vivo* activity of AHVB predicted it to be significantly less on the basis of *in vitro* cytotoxicity studies.

**Patient Studies**

In preclinical studies involving human tumor xenografts of non-small cell lung cancer (NSCLC) and cervical cancer, AHVB showed superior activity to that of both vincristine

and vinorelbine at equitoxic doses. Toxicologic studies in rats and dogs demonstrated reversible myelosupression and gastrointestinal toxicities. Based on these data, a Phase I trial was undertaken to determine the feasibility of administering AHVB, as a 1 hr intravenous (IV) infusion once every 3 weeks, to patients with advanced refractory solid tumors, to determine the maximum-tolerated dose (MTD), the dose limiting toxicity (DLT) and to evaluate the major pharmacokinetic parameters.

Patients had normal bone marrow, hepatic and renal function. Twenty-four patients were treated with escalating doses of AHVB, given as a 1 hour infusion every 3 weeeks. Twenty-one of the 24 patients were evaluable. There were 12 male and 12 female patients with a median age of 60 years (range 27-75). Diagnoses were non-small cell lung cancer (NSCLC), colorectal cancer, soft tissue sarcoma, pancreatic cancer, breast cancer and metastatic neuroendocrine cancer in 11, 5, 4, 1, 1, and 1 patient(s), respectively. Patients have had a median of 3 chemotherapy regimens (range 1-6). A total of 51 courses were administered at doses of 2.5, 5, 10, 16.5, 21, 25 and 30 mg/m$^2$ to 1, 3, 1, 3, 6, 6 and 1 patient(s), respectively (See Table 4).

| Dose Level | Dose Concentration (mg/m$^2$) | Patient(s) treated |
|---|---|---|
| 1 | 2.5 | One patient treated |
| 2 | 5.0 | One patient treated developed Grade 2 elevated amylase, elevated creatine and anorexia requiring an additional 2 patients to be added at this dose |
| 3 | 10 | One patient treated with minimal toxicity |
| 4 | 16.5 | One patient treated expired 4 days after beginning cycle due to disease process (unrelated to drug study). The patient was replaced by another patient which was found to have brain metastasis requiring radiation therapy and was replaced. The next patient showed Grade 2 toxicities (anemia, hypertension, tachycardia, diaphoresis, flushing and fatigue) requiring 2 more patients at this dose. These two patients had minimal toxicities. |
| 5 | 25 | Six patients were treated with 2 having DLTs in the form of grade 4 constipation, Grade 3 tumor pain, nausea, vomiting, anemia and Grade 4 neutropenia (1 patient). |
| 6 | 30 | One patient treated developed Grade 3 leukopenia. |

| | | |
|---|---|---|
| | | Since minimal toxicity was seen at 16.5 mg/m$^2$ dose level and the increment from 16.5 to 25 mg/m$^2$ represented a 50% increase, it was elected to evaluate an intermediate dose level at 21 mg/m$^2$. |
| 7 | 21 | Seven patients enrolled but only 6 were evaluable for toxicity. One patient was replaced since treatment was incomplete and non-evaluable. At this dose level, one patient exhibited Grade 3 nausea and vomiting. This does was determined to be the MTD and the study was closed. |

Table 4: Patient dosage levels and associated toxicities.

The first patient was entered at the 2.5 mg/m$^2$ dose level. At the next dose level (5 mg/m$^2$), three patients were enrolled because the first patient had non dose limiting toxicities in the form of Grade 2 anorexia, hyperamylasemia, and increased serum creatinine. Five patients were enrolled at the 16.5 mg/m$^2$ dose level because two patients were not evaluable and had to be replaced. Grade 2 toxicities including infusional hypertension, anemia and dizziness were noted at the 16.5 mg/m$^2$ dose level. Six patients were enrolled at the 25 mg/m$^2$ dose level. DLTs including Grade 4 level constipation and Grade 3 nausea and vomiting were noted at this dose level. This dose level exceeded the MTD. Since minimal toxicities were seen at the 16.5 mg/m$^2$ dose level and the increment from 16.5 to 25 mg represented a 50% increase, it was elected to evaluate an intermediate does level of 21 mg/m$^2$. Seven patients were enrolled at the 21 mg/m$^2$ dose level, but only 6 were evaluable for toxicity; one patient developed grade 2 hypertension, headache, nausea and vomiting at the start of the infusion, that recurred on rechallenge. This patient received only 15 ml of the drug solution and was not evaluable to assess toxicity. One patient at this dose level had Grade 3 nausea and vomitting and Grade 2 constipation, requiring brief hospitalization, laxatives and IV fluids.

The nausea and vomiting at the 21 mg/m$^2$ dose level was a DLT. It was determined that this dose is the MTD. Stable disease was noted at a dose level of 10 mg/m$^2$ in one patient with metastatic sarcoma to lungs and at dose levels of 21 and 25 mg/m$^2$ in three patients with metastatic NSCLC.

AHVB blood serum concentrations were measured at 0, 0.33, 0.67,1, 1.25, 1.5, 2, 4, 6, 8, 12, 24,48, and 72 hr after the start of each infusion. Figures 10 shows a time-course

plot of individual patient AHVB serum concentrations. The serum extracts were assayed using high pressure liquid chromatography (HPLC) and were fit to a 2-compartment pharmacokinetic model for determination of pharmacokinetic parameters (See Table 5).

| Dose ($mg/m^2$) | | A (ng/mL) | $T_{1/2}$ al (h) | AUC (ug/mL*h) | B (ng/mL) | $T_{1/2}$ be (h) | CL ($L/h/m^2$) | $C_{max}$ (ng/mL) | $V_{ss}$ ($L/m^2$) |
|---|---|---|---|---|---|---|---|---|---|
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Value | 134.71 | 0.12 | 71.64 | 5.03 | 6.63 | 34.90 | 28.23 | 226.04 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Mean | 281.82 | 0.14 | 571.75 | 8.04 | 44.36 | 10.61 | 51.37 | 534.05 |
| | Min | 135.55 | 0.07 | 282.20 | 6.27 | 27.54 | 6.30 | 38.65 | 445.34 |
| | Median | 178.39 | 0.17 | 639.67 | 8.11 | 42.41 | 7.82 | 53.07 | 534.71 |
| | Max | 531.52 | 0.17 | 793.39 | 9.73 | 63.13 | 17.72 | 62.40 | 622.10 |
| | CV% | 77.11 | 41.87 | 45.87 | 21.53 | 40.30 | 58.42 | 23.28 | 16.55 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Value | 109.85 | 0.25 | 247.19 | 11.49 | 12.50 | 40.45 | 48.57 | 614.22 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Mean | 509.47 | 0.22 | 824.58 | 22.50 | 21.35 | 25.13 | 163.03 | 486.18 |
| | Min | 263.49 | 0.13 | 511.26 | 6.64 | 8.21 | 9.21 | 96.35 | 298.06 |
| | Median | 478.82 | 0.20 | 541.21 | 21.87 | 21.82 | 30.49 | 171.48 | 485.95 |
| | Max | 816.42 | 0.39 | 1791.22 | 34.16 | 33.52 | 32.27 | 231.52 | 625.73 |
| | CV% | 41.58 | 46.33 | 66.79 | 51.82 | 47.25 | 36.55 | 30.17 | 28.34 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Mean | 774.37 | 0.26 | 1094.91 | 25.92 | 22.88 | 19.87 | 257.21 | 488.51 |
| | Min | 322.90 | 0.19 | 918.58 | 24.50 | 19.00 | 14.94 | 179.07 | 471.27 |
| | Median | 802.43 | 0.20 | 964.03 | 24.72 | 19.15 | 21.78 | 252.10 | 486.76 |
| | Max | 1197.78 | 0.39 | 1402.11 | 28.54 | 30.50 | 22.86 | 340.46 | 507.51 |
| | CV% | 56.58 | 43.26 | 24.39 | 8.76 | 28.83 | 21.51 | 31.42 | 3.72 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Mean | 1122.91 | 0.18 | 764.83 | 34.51 | 10.68 | 36.43 | 273.43 | 380.18 |
| | Min | 379.79 | 0.11 | 436.85 | 20.78 | 6.73 | 20.80 | 108.52 | 227.92 |
| | Median | 1062.6 | 0.16 | 750.85 | 35.06 | 10.54 | 33.48 | 287.71 | 270.11 |
| | Max | 1952.78 | 0.36 | 1202.01 | 48.76 | 16.29 | 57.23 | 368.79 | 762.66 |
| | CV% | 59.00 | 49.42 | 35.74 | 32.63 | 33.27 | 36.30 | 34.30 | 55.41 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| | Value | 3559.02 | 0.10 | 1797.92 | 36.71 | 23.83 | 16.69 | 571.08 | 403.55 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |

| | | | | |
|---|---|---|---|---|
| **Mean** | 0.19 | 20.8 | 26.4 | 457 |
| **Min** | 0.07 | 6.63 | 6.30 | 226 |
| **Median** | 0.17 | 17.60 | 25.30 | 479 |
| **Max** | 0.393 | 63.10 | 57.30 | 763 |
| **CV%** | 47 | 68 | 49 | 34 |

Table 5: Anhydrovinblastine Pharmacokinetic Parameters.

The pharmacokinetics of AHVB are linear, and well characterized by a 2-compartment model, with a mean clearance of 26.4 L/h/m$^2$; alpha half-life 0.19 h; beta half-life 20.8 h; $V_{ss}$ 451 L/m$^2$. There appears to be no significant change in clearance observed between smaller and larger doses ($p > 0.2$ by linear regression). Figure 11 shows the clearance of AHVB for each patient at their respective dose level. Similarly, AUC appeared to increase linearly with dose ($r^2 = 0.82$, $p < 0.05$) (Fig. 12). A similar finding was observed with the maximum plasma concentration ($C_{max}$) (Fig. 13). The variability in clearance and volume of distribution was modest, with a CV% of 49% and 39%, respectively.

Vinblastine has been reported to have a triphasic elimination profile, with the initial alpha half-life of less than 5 minutes. The current study precluded an evaluation of this rapid phase, if it exists for AHVB. Otherwise the pharmacokinetics of AHVB were similar to that reported for vinblastine.

**Pharmaceutical Preparations**

The present invention also provides pharmaceutical compositions containing a compounds as disclosed in the claims in combination with one or more pharmaceutically acceptable, inert or physiologically active, diluents or adjuvants. The compounds of the invention can be freeze dried and, if desired, combined with other pharmaceutically acceptable excipients to prepare formulations for administration. These compositions may be presented in any form appropriate for the administration route envisaged. In one embodiment of the invention, parenteral and the intravenous preparations of AHVB are used.

3',4'-anhydrovinblastine may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition, there is provided a pharmaceutical formulation comprising 3',4'-anhydrovinblastine and a pharmaceutically acceptable carrier. 3',4'-anhydrovinblastine may be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants and if desired other active ingredients. The pharmaceutical compositions containing 3',4'-anhydrovinblastine may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion hard or soft capsules, or syrups or elixirs.

Compositions intended of oral use may be prepared according to any known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate: granulating and disintegrating agents for example, corn starch, or alginic acid: binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia: dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethyene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or

wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oils phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. this suspension may be formulation according to known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

3',4'-anhydrovinblastine may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug

with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

3',4'-anhydrovinblastine may be administered parenterally in sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anaesthetics, preservatives and buffering agents can be dissolved in the vehicle.

**Administration of AHVB**

For AHVB, the dose to be administered, whether a single dose, multiple dose, or a daily dose, will vary and a dose regimen is designed based on the potency of the compound, route of administration, size of the recipient and the nature of the patient's condition.

The dosage to be administered is not subject to defined limits, but in will usually be an effective amount. It will usually be the equivalent, on a molar basis of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active free drug to achieve its desired pharmacological and physiological effects.

An oncologist skilled in the art of cancer treatment will be able to ascertain, without undue experimentations, appropriate protocols for effective administration of the compounds of this present invention by referring to the earlier studies of vinblastine and its derivatives.

AHVB may be administered in a number of ways depending upon whether local or systemic treatment of the organism is desired. Administration may be pulmonary, *e.g.* by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or

intramuscular injection or infusion; or intracranial, *e.g.* intrathecal or intraventricular, administration. AHVB may be administered topically in a lotion or cream, for application to the skin in order to treat for example a melanoma.

For administration to an individual for the treatment cancer, the present invention also contemplates the formulation of AHVB into oral dosage forms such as tablets, capsules and the like. For this purpose, pharmaceutical compositions comprising AHVB can be combined with conventional carriers, such as magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethyl-cellulose, low melting wax, cocoa butter and the like. Diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, tablet-disintegrating agents and the like can also be employed, if required. AHVB or pharmaceutical compositions comprising AHVB can be encapsulated with or without other carriers. In all cases, the proportion of active ingredients in any solid and liquid composition will be at least sufficient to impart the desired activity to the individual being treated upon oral administration. The present invention further contemplates parenteral injection of AHVB or pharmaceutical compositions comprising AHVB, in which case they are used in the form of a sterile solution containing other solutes, for example, enough saline or glucose to make the solution isotonic.

For administration by inhalation or insufflation, AHVB or pharmaceutical compositions comprising AHVB can be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol.

The dosage requirements for AHVB or pharmaceutical compositions comprising AHVB vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Dosage requirements can be determined by standard clinical techniques, known to a worker skilled in the art. Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached. In general, AHVB or pharmaceutical

compositions comprising AHVB is administered at a concentration that will generally afford effective results while minimizing harmful or deleterious side effects. Administration can be either as a single unit dose or, if desired, the dosage can be divided into convenient subunits that are administered at suitable times throughout the day. In an embodiment of the invention intravenous dosages ranging from 2.5 mg/m$^2$ to 30 mg/ m$^2$ may be administered to patients over 1 hour once every 3 weeks. This regimen may repeated for 51 courses of treatment.

**Therapeutic Uses**

AHVB, a derivative of the Vinca Alkaloid Vinblastine, has shown significant cytotoxic potential against a panel of human cancer cell lines, and significant activity against the human H460 non-small cell lung carcinoma tumour xenograph in SCID/Rag-2 Mice. *In vitro* cytotoxicity assays utilizing the MTT cytotoxicity assay with a drug exposure time of 72 hours have shown that AHVB is an active cytotoxic drug with $IC_{50}$ values ranging from 20-24 nM against the H460 human non-small cell lung carcinoma, C-4 human cervical carcinoma, K562 human leukemia, and the A431 human epidermoid cell lines. AHVB was approximately 10-fold less active than Navelbine™ when tested *in vitro* against the same cell lines. Surprisingly, however when AHVB was tested *in vitro* in solid tumour efficacy experiments it was found to be more potent than Navelbine™. Male SCID/Rag-2 mice were inoculated sc. with H460 cells and after 12 days of tumour growth AHVB and Navelbine™ were delivered i.v. at doses of 10mg/kg and 20 mg/kg on days 1, 5, 9. In this model, AHVB caused greater tumour growth inhibition and was less toxic than Navelbine™. These results suggest that AHVB may have desirable pharmacological properties for therapeutic applications.

AHVB may be used as part of a neo-adjuvant therapy (to primary therapy), as part of an adjuvant therapy regimen, or also for the treatment of locally advanced and metastatic disease.

Primary therapy is understood to encompass a first line of treatment upon the initial

diagnosis of cancer in a patient. Exemplary primary therapies may involve surgery, a wide range of chemotherapies and radiotherapy.

Adjuvant therapy is understood to encompass any therapy, following a primary therapy such as surgery, that is administered to patients at risk of relapsing. Adjuvant systemic therapy is begun soon after primary therapy to delay recurrence, prolong survival or cure a patient. It is contemplated that AHVB can be used in further combination with other chemotherapeutic agents as part of an adjuvant therapy.

In the application of cancer therapies a patient's response status is monitored and refers to measuring what happens to the tumour(s) or lesion(s) under chemotherapy, namely any observed growth (progression of disease), stability, or shrinkage (complete or partial response). Arising out such monitoring may be the observation of relapse in a patient which may refer to the relapse of a patient with advanced disease. Relapse time is the time from the initial appearance of a primary cancer to the appearance of advanced disease requiring chemotherapy.

The progression of advanced disease is monitored to help evaluate when chemotherapy, may be appropriate and may be marked by an increase of at least 25% in the overall sum of measurable lesions as compared to nadir (*i.e.* best response) and/or the appearance of new lesions following primary therapy. Alternatively, lesions may be found to shrink in size.

In an embodiment of the invention a 1 hr IV infusion of AHVB, at doses ranging from 2.5 $mg/m^2$ to 30 $mg/m^2$, once every 3 weeks for 51 courses to patients with advanced refractory solid tumors is used. In another embodiment of the invention treatment by IV infusion of AHVB for 1 hour, every 3 weeks for up to 51 is administered at doses of 10 $mg/m^2$ to 25 $mg/m^2$.

It is to be understood that the examples described above are not meant to limit the scope of the present invention. It is expected that numerous variants will be obvious to

the person skilled in the art to which the present invention pertains, without any departure from the spirit of the present invention. The appended claims, properly construed, form the only limitation upon the scope of the present invention.

# CLAIMS

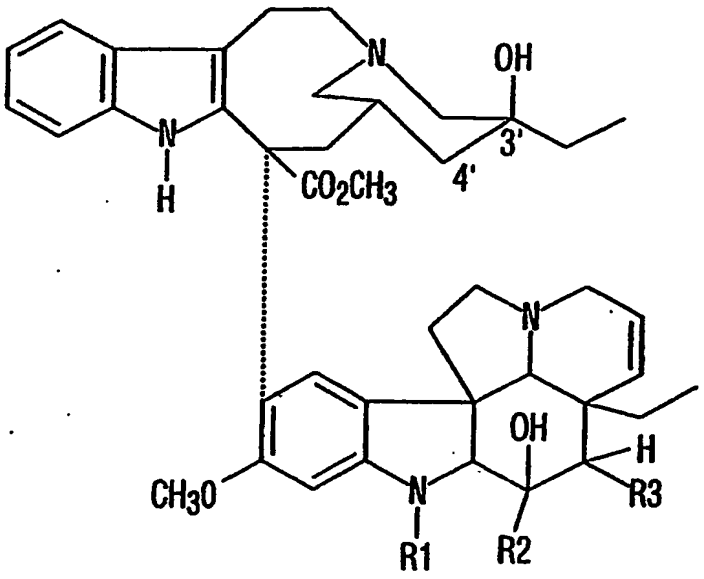
We claim:

1. The use of 3',4'-anhydrovinblastine, or variants thereof, as an antineoplastic agent in the treatment of cancer.

2. The use according to claim 1, wherein the cancer is a solid tumor.

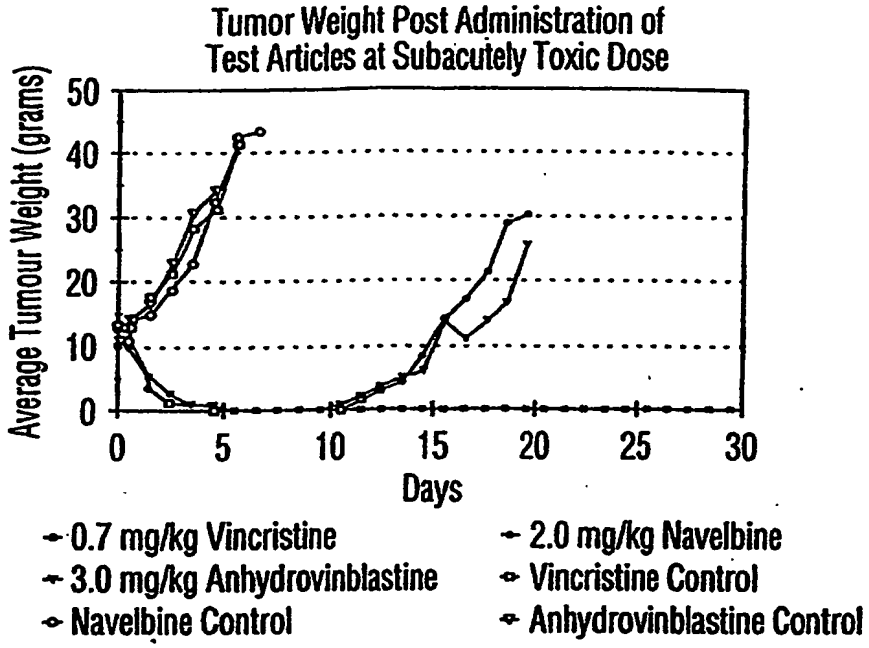
3. The use according to claim 2, wherein said solid tumor is a sarcoma or a carcinoma.

4. The use of 3',4'-anhydrovinblastine at a dosage concentration of 2.5 to 30 $mg/m^2$ to treat a cancer in a mammal.




| | R1 | R2 | R3 |
|---|---|---|---|
| Vindesine | $-CH_3$ | $-CONH_2$ | -OH |
| Vincristine | -CHO | $-CO_2CH_3$ | $-OCOCH_3$ |
| Vinblastine | $-CH_3$ | $-CO_2CH_3$ | $-OCOCH_3$ |

FIG. 1


Tumor Weight Post Administration of Test Articles at Subacutely Toxic Dose
Average Tumour Weight (grams)
50
40
30
20
10
0
0
5
10
15
20
25
30
Days
0.7 mg/kg Vincristine
2.0 mg/kg Navelbine
3.0 mg/kg Anhydrovinblastine
Vincristine Control
Navelbine Control
Anhydrovinblastine Control

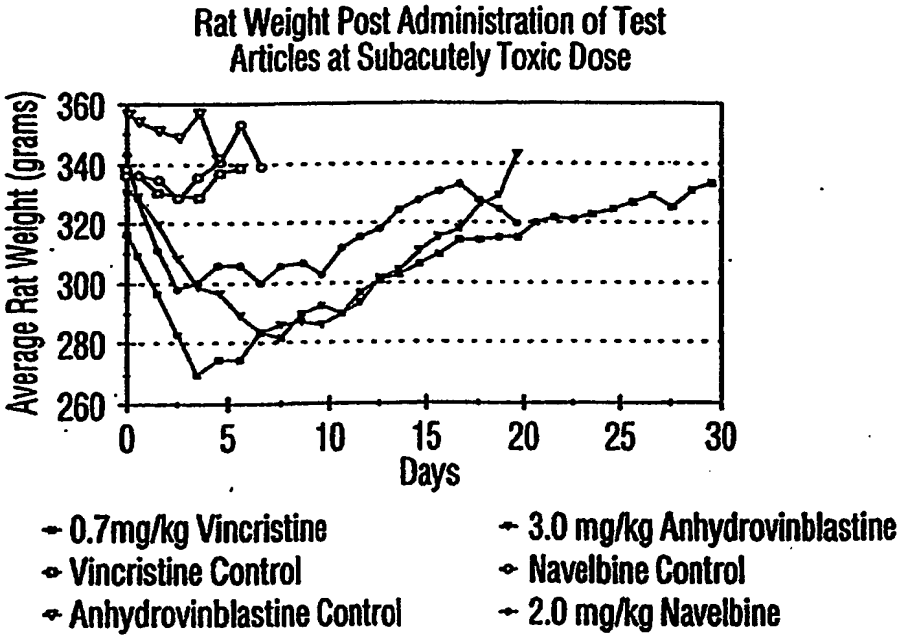

Rat Weight Post Administration of Test Articles at Subacutely Toxic Dose
Average Rat Weight (grams)
360
340
320
300
280
260
0
5
10
15
20
25
30
Days
0.7mg/kg Vincristine
3.0 mg/kg Anhydrovinblastine
Vincristine Control
Navelbine Control
Anhydrovinblastine Control
2.0 mg/kg Navelbine

FIG. 2

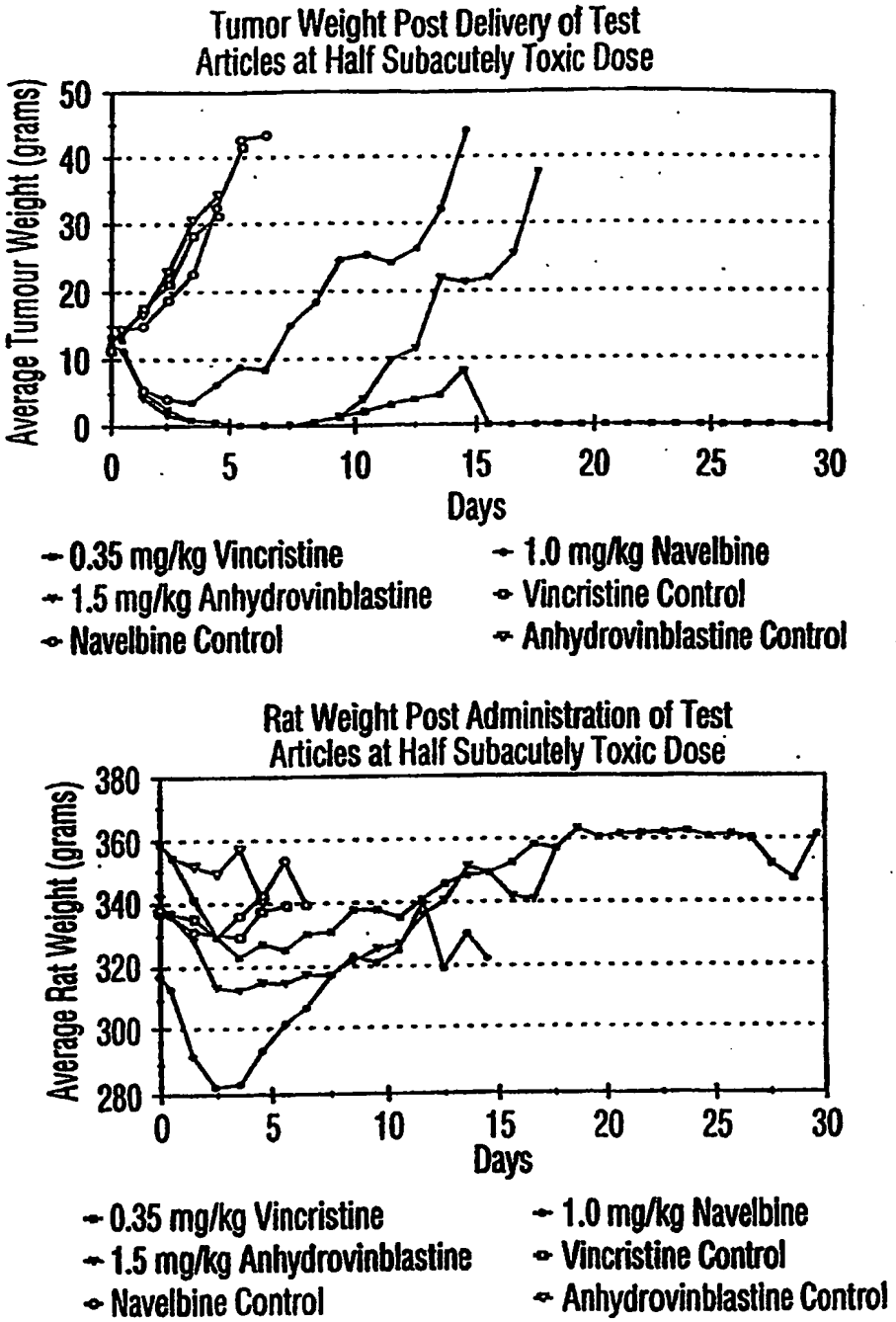

Tumor Weight Post Delivery of Test Articles at Half Subacutely Toxic Dose
Average Tumour Weight (grams)
50
40
30
20
10
0
0
5
10
15
20
25
30
Days
0.35 mg/kg Vincristine
1.5 mg/kg Anhydrovinblastine
Navelbine Control
1.0 mg/kg Navelbine
Vincristine Control
Anhydrovinblastine Control
Rat Weight Post Administration of Test Articles at Half Subacutely Toxic Dose
Average Rat Weight (grams)
380
360
340
320
300
280
0
5
10
15
20
25
30
Days
0.35 mg/kg Vincristine
1.5 mg/kg Anhydrovinblastine
Navelbine Control
1.0 mg/kg Navelbine
Vincristine Control
Anhydrovinblastine Control

FIG. 3

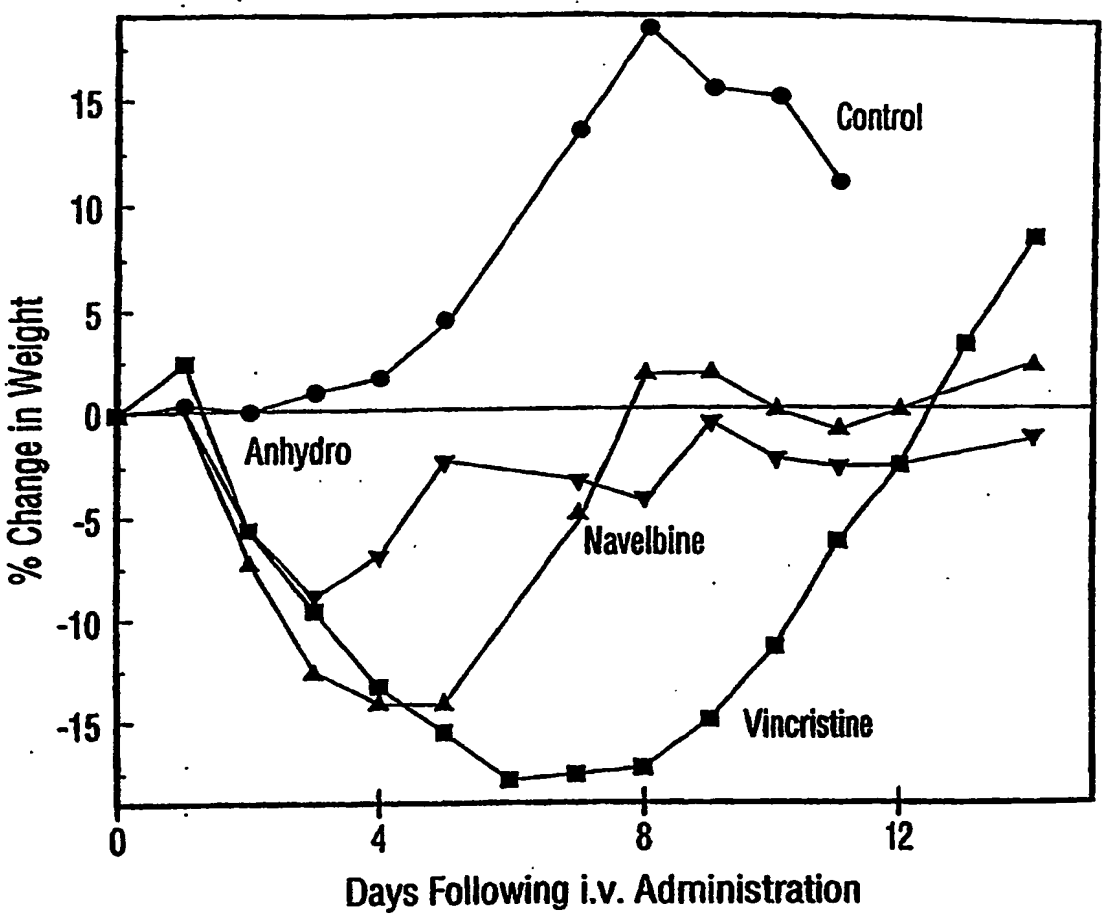

15
10
5
0
-5
-10
-15
% Change in Weight
Control
Anhydro
Navelbine
Vincristine
0
4
8
12
Days Following i.v. Administration

FIG. 4

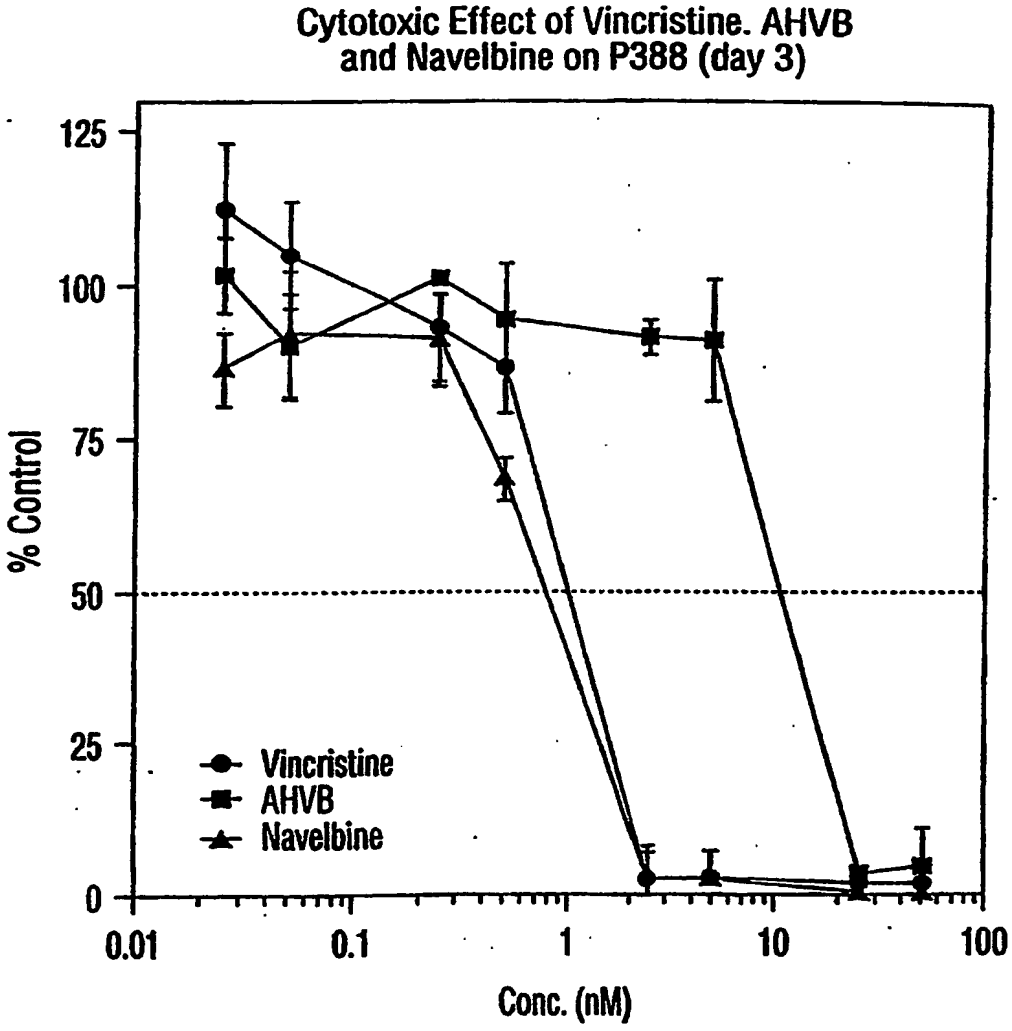

Cytotoxic Effect of Vincristine. AHVB and Navelbine on P388 (day 3)
125
100
75
50
25
0
% Control
Vincristine
AHVB
Navelbine
0.01
0.1
1
10
100
Conc. (nM)

FIG. 5

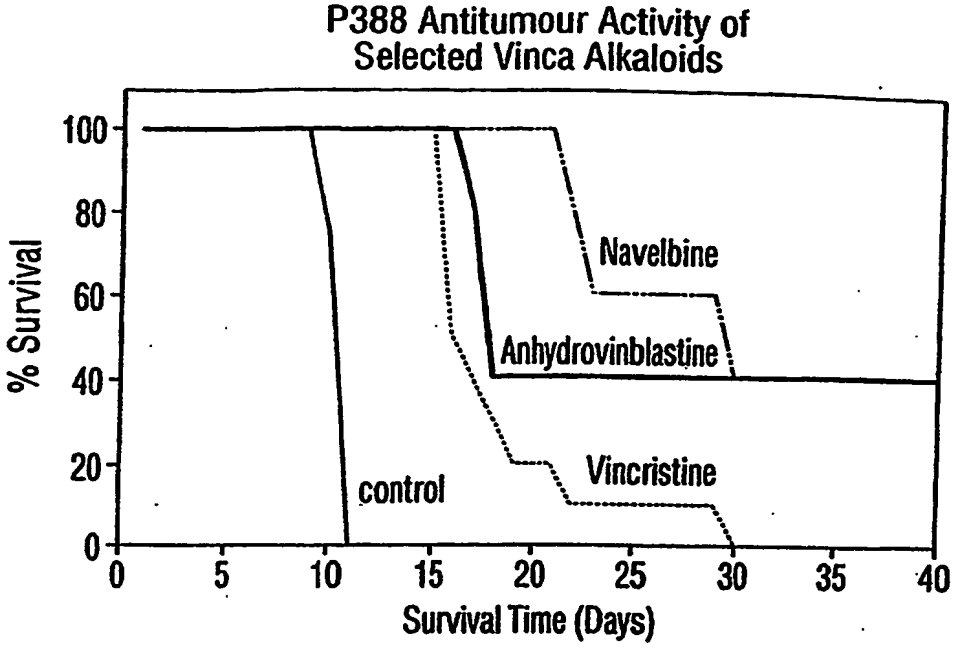

P388 Antitumour Activity of
Selected Vinca Alkaloids
% Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Survival Time (Days)
Navelbine
Anhydrovinblastine
Vincristine
control

FIG. 6

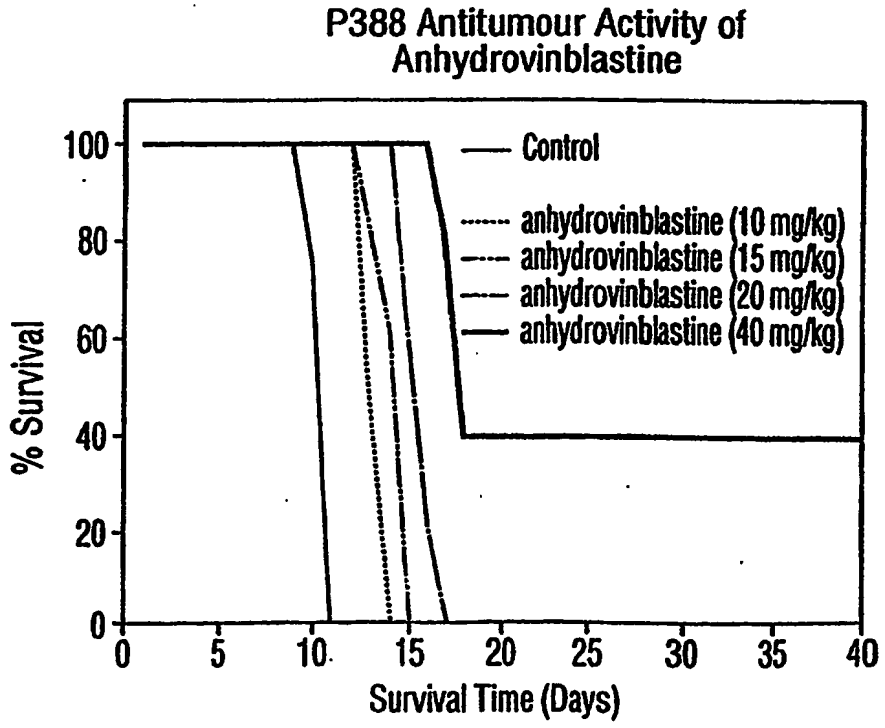

P388 Antitumour Activity of
Anhydrovinblastine
% Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Survival Time (Days)
Control
anhydrovinblastine (10 mg/kg)
anhydrovinblastine (15 mg/kg)
anhydrovinblastine (20 mg/kg)
anhydrovinblastine (40 mg/kg)

FIG. 7

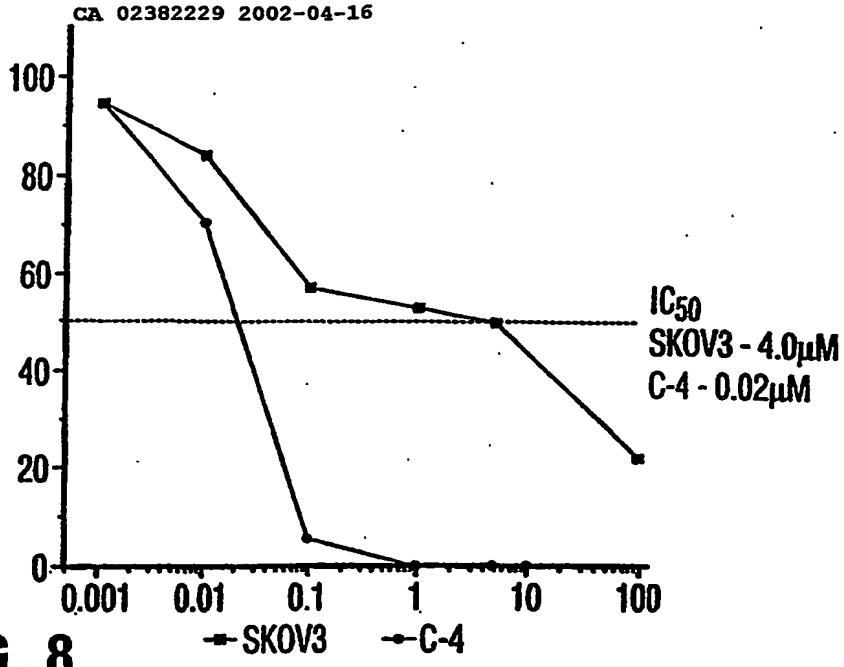



FIG. 8

SKOV3 - human ovarian cancer
C-4 - human cervical carcinoma

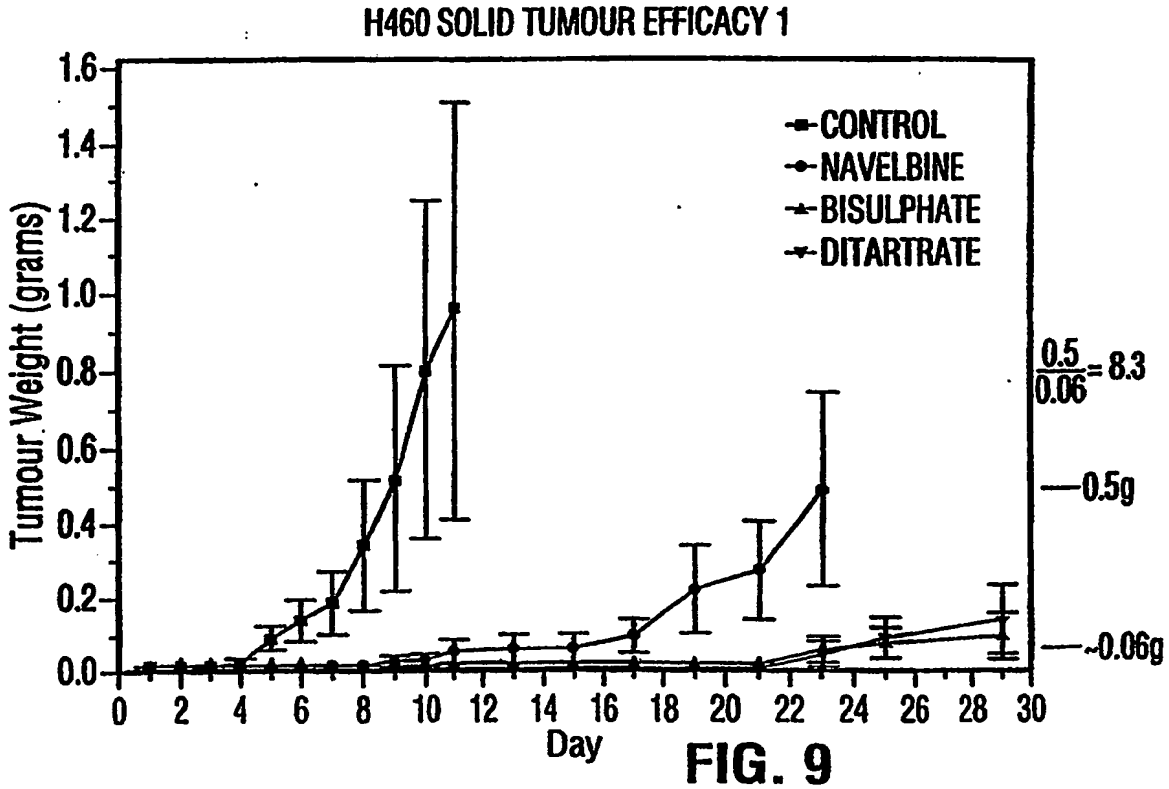
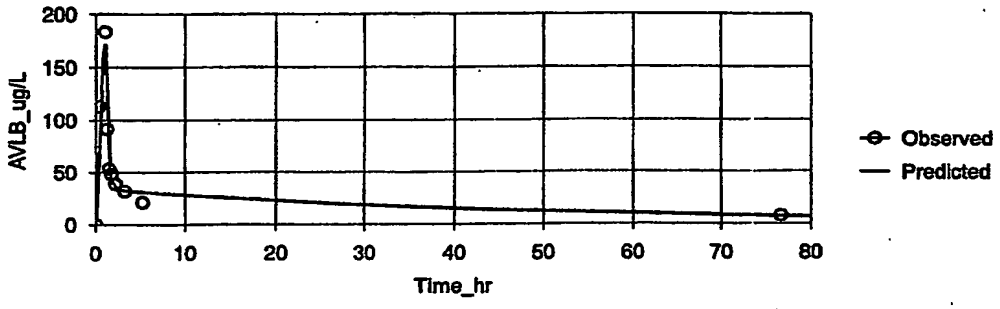


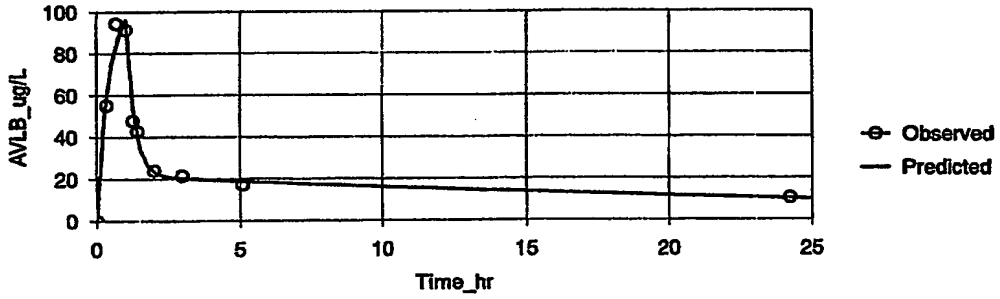

FIG. 9

# INDIVIDUAL PATIENT MODEL FITS

**Patient 1**




**Patient 2**




**Patient 3**

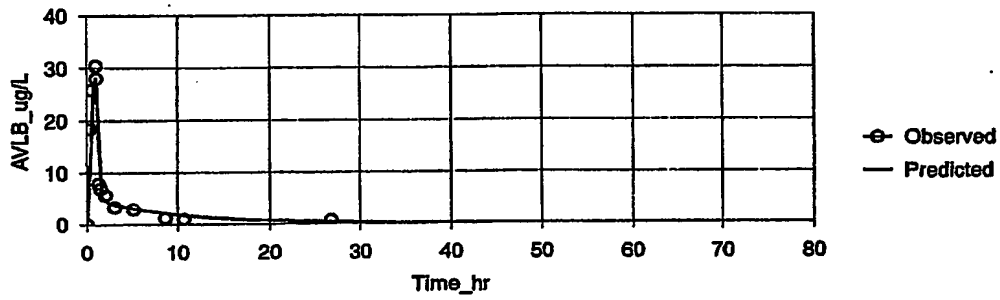
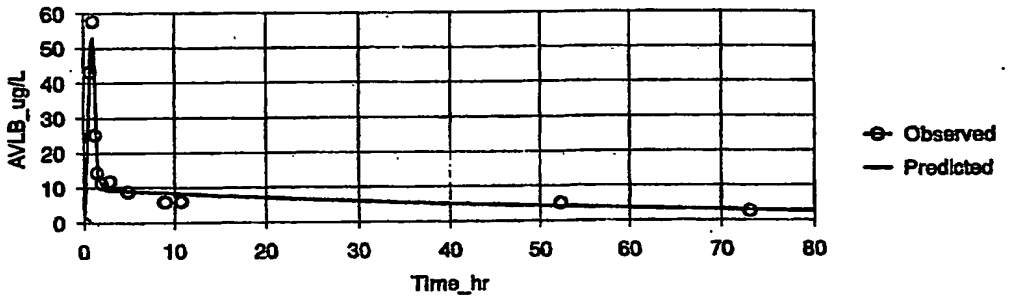
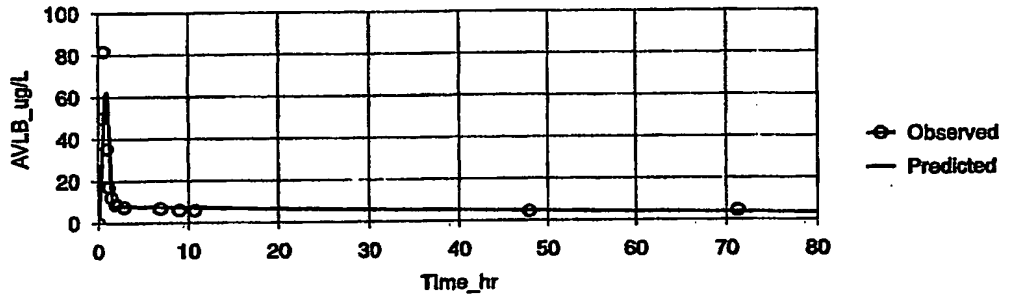



**Figure 10**

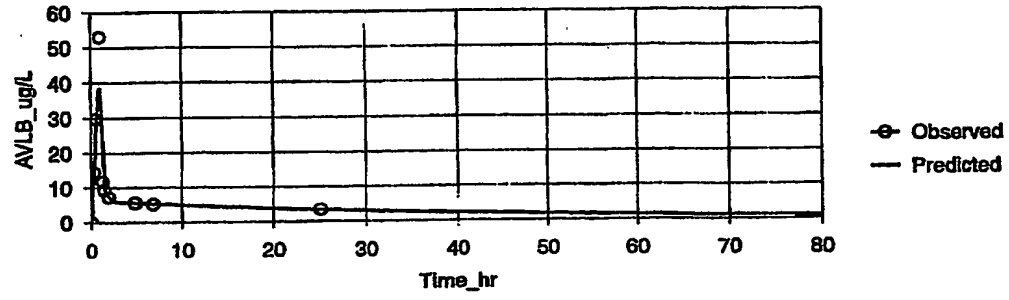
**Patient 4**



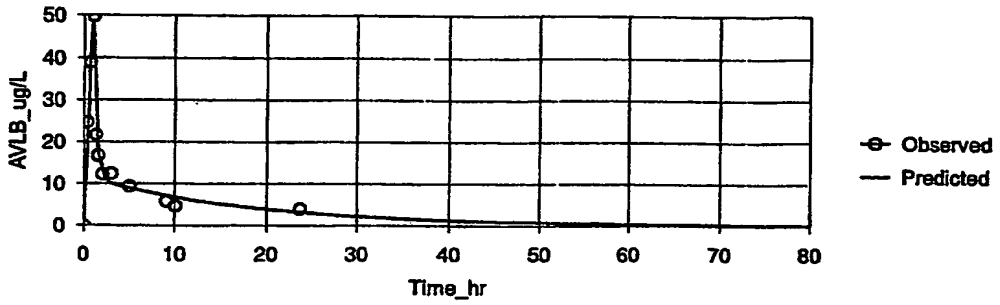

**Patient 5**



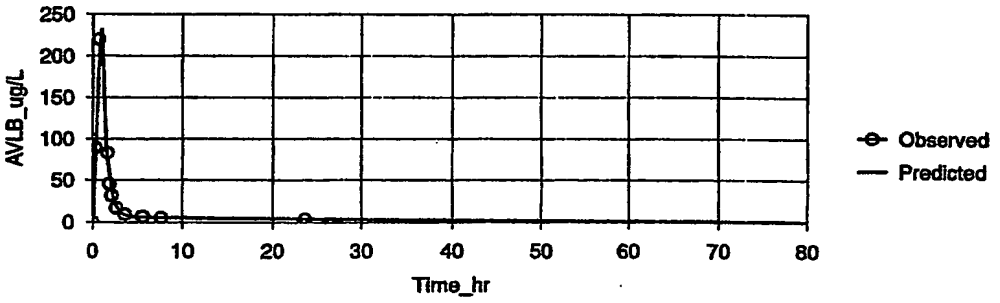

**Patient 6**



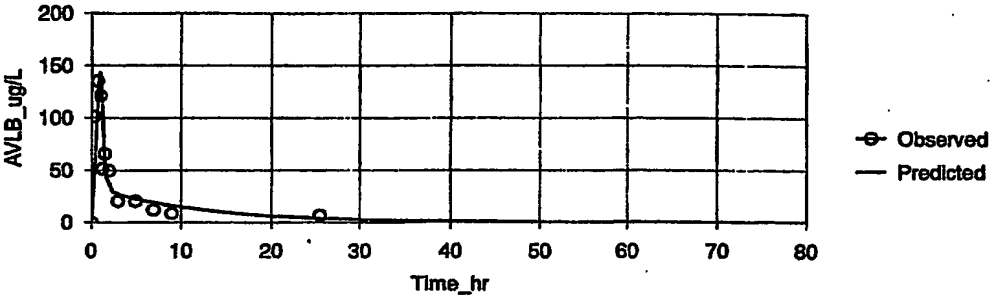
Patient 7



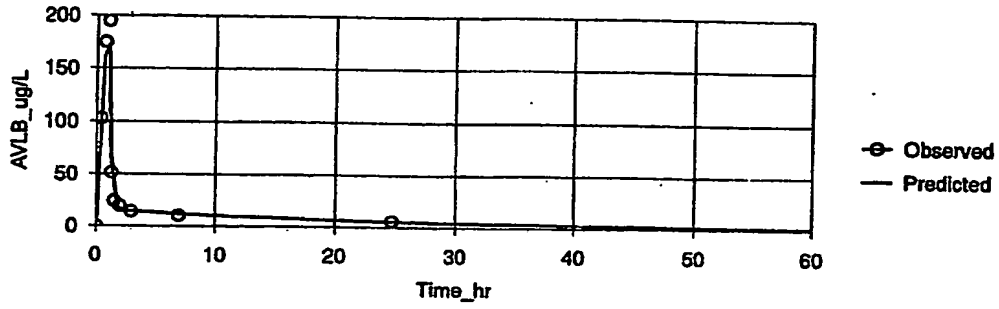

Patient 8



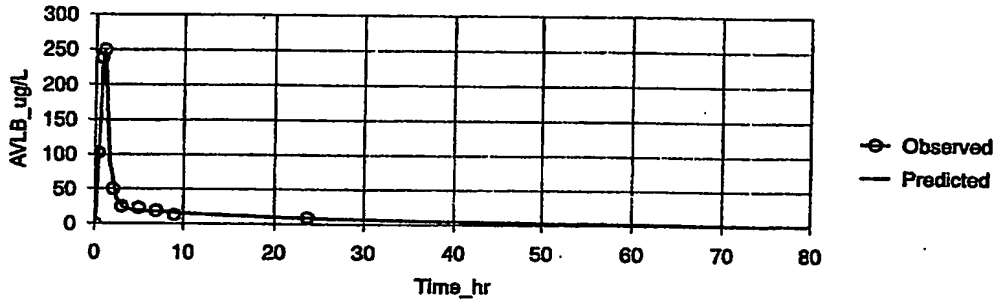

Patient 9



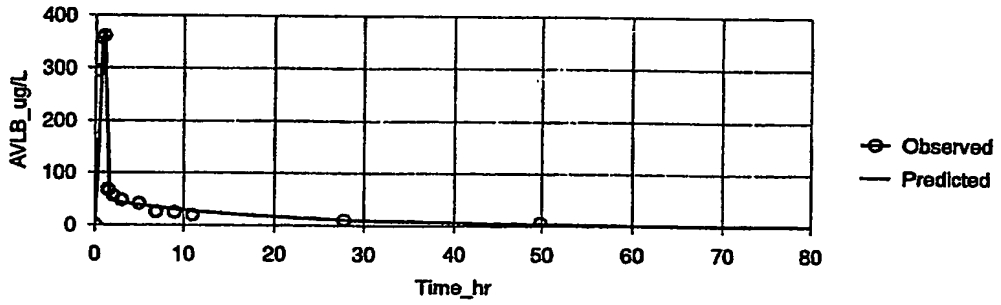
**Patient 10**



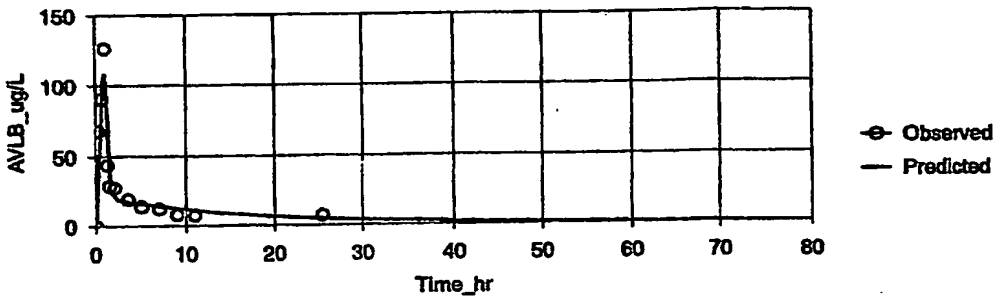

**Patient 11**



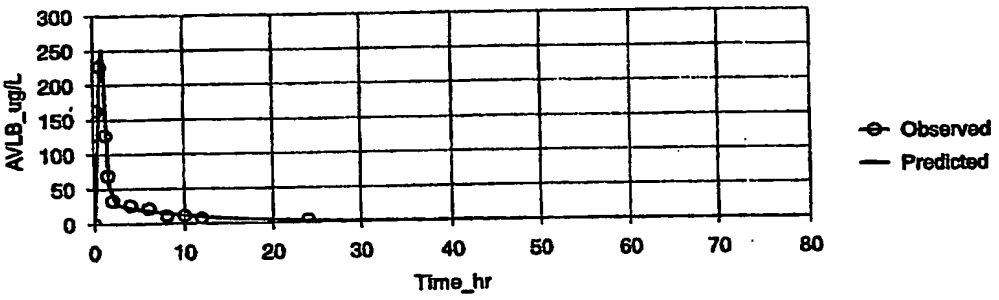

**Patient 12**



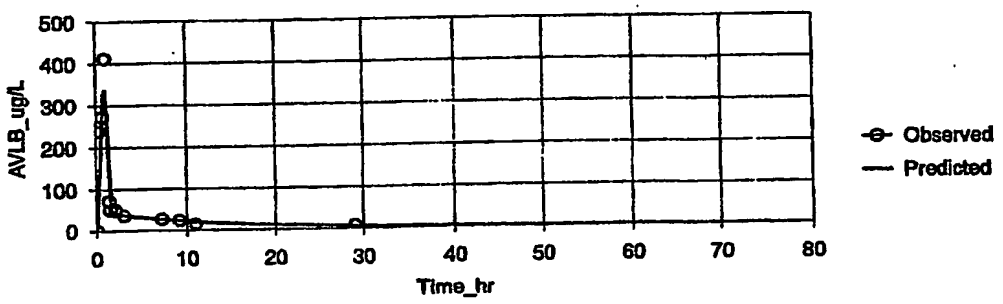
Patient 13



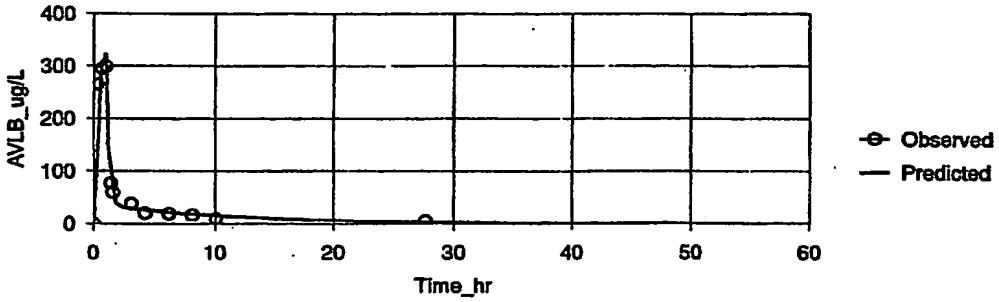

Patient 14



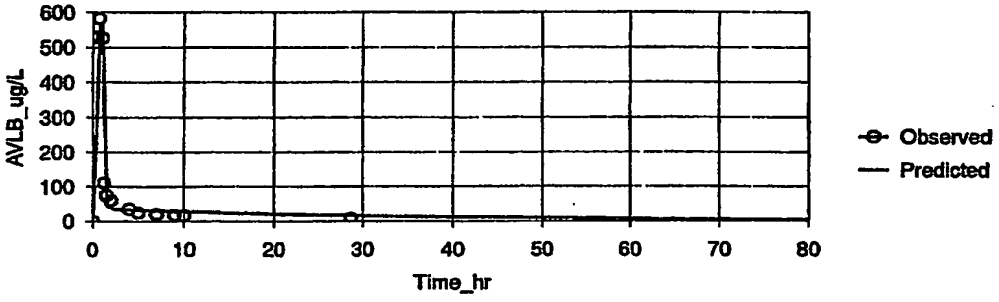

Patient 15



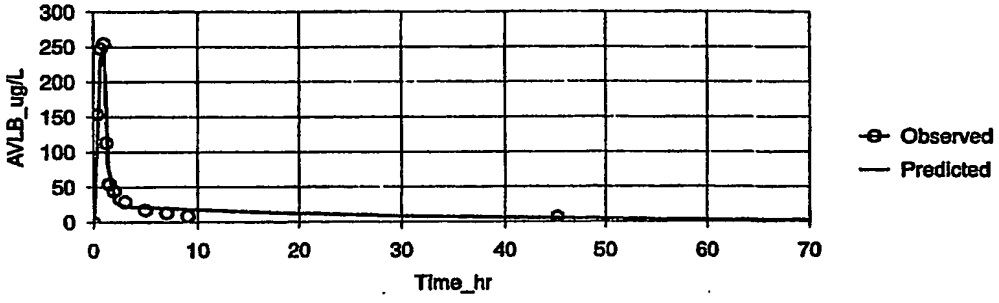
**Patient 16**



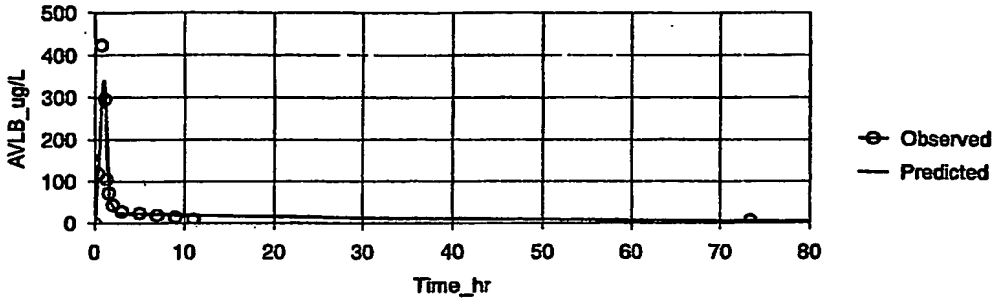

**Patient 17**



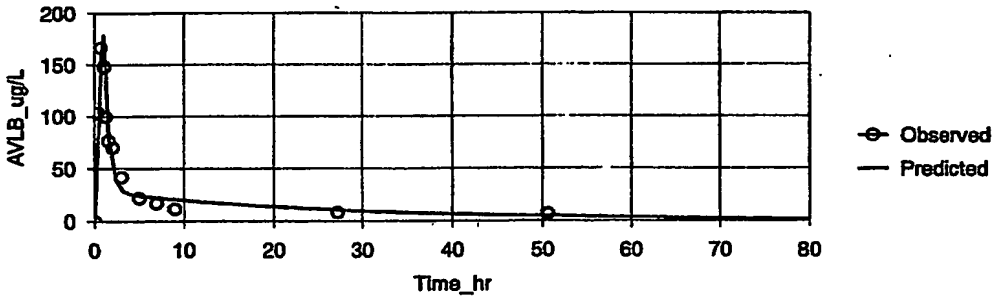

**Patient 18**



Patient 19




Patient 20



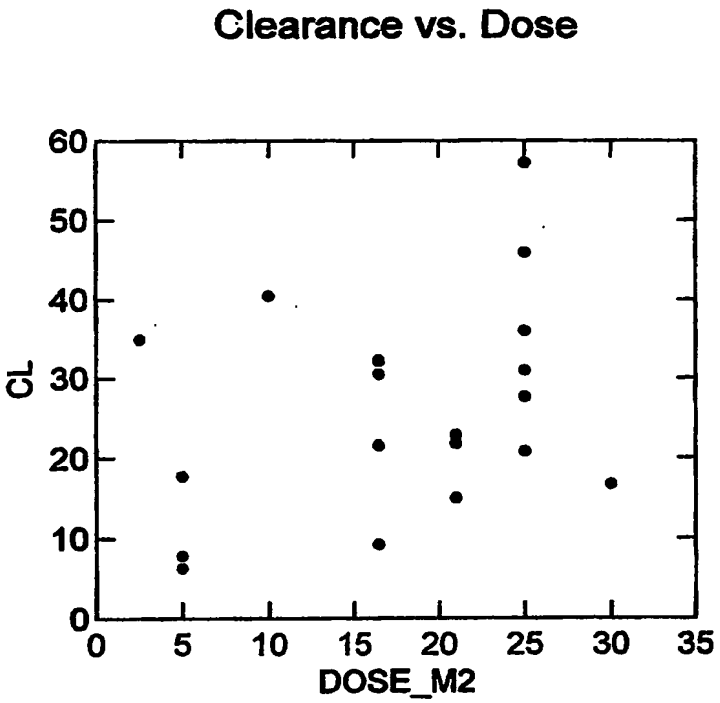



Figure 11

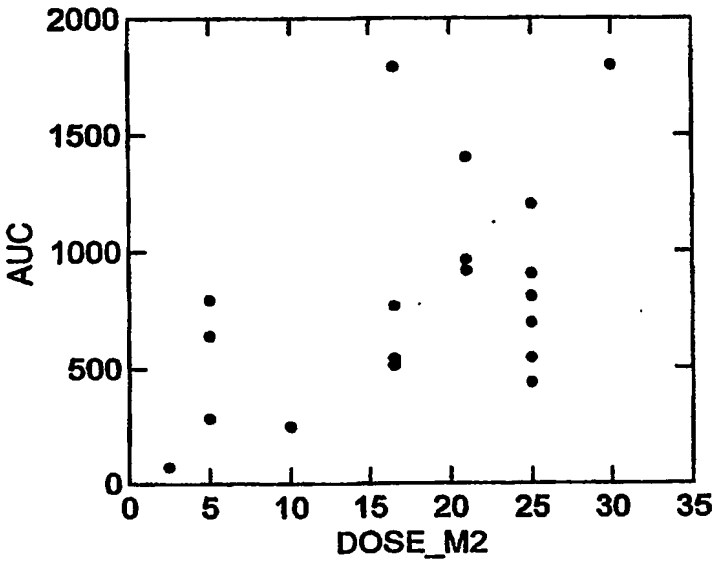



Figure 12

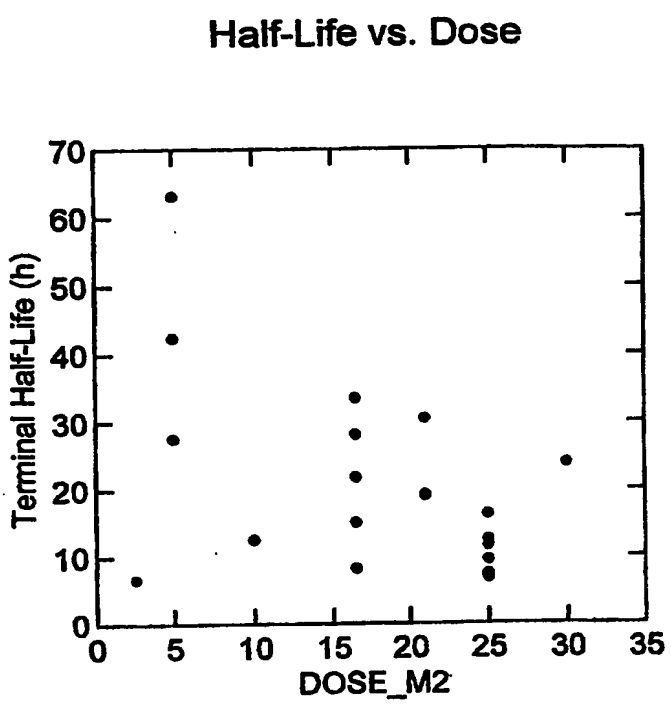



Figure 13

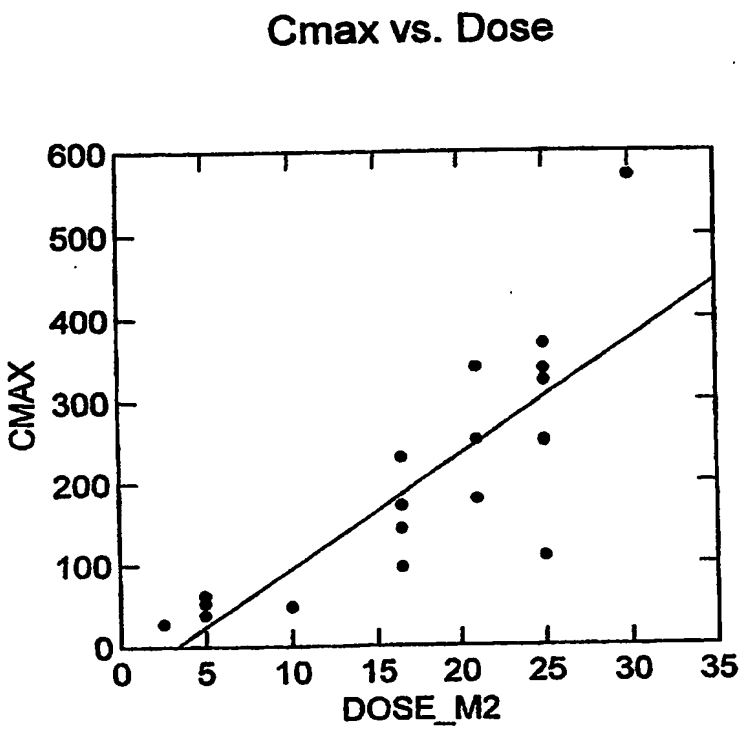



Figure 14

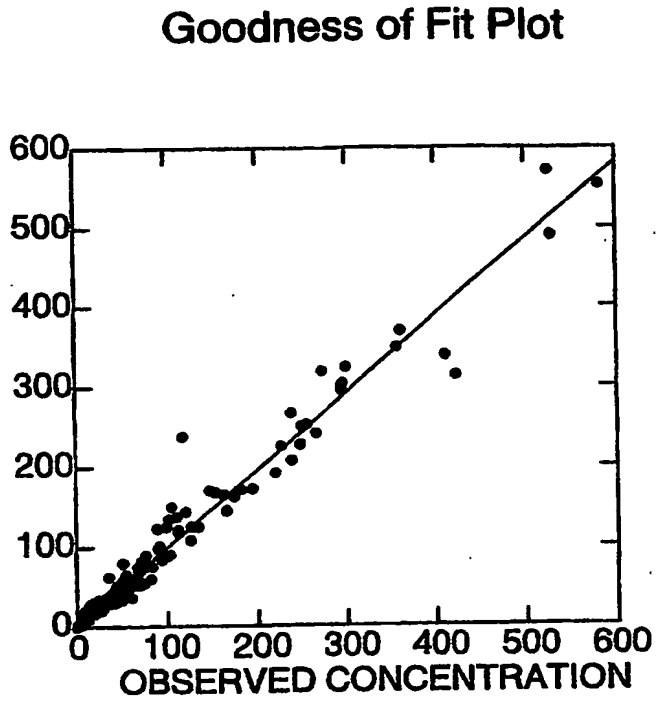

Goodness of Fit Plot
600
500
400
300
200
100
0
0
100
200
300
400
500
600
OBSERVED CONCENTRATION

Figure 15